HISTOPLASMOSIS

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Edited by

HENRY C SWEANY, M D

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This book is affectionately dedicated
to the memory of my late wife

Ethyle M Sweany

PREFACE

There is not the least doubt about the propriety and need for a monograph on histoplasmosis. A disease that has rocketed to the forefront of medicine as has histoplasmosis within the last twelve years demands a clear, concise, authoritative and all-inclusive exposition of the subject. This disease has so mimicked other diseases, especially tuberculosis, that it has caused much confusion in diagnosis and treatment of patients in the past. The medical profession will be eager to find out more about this disease that has so many perfect masquerades. It is hoped that this book will achieve the intended goal and help to clear up some of the confusion that has existed.

Credit for initiating this project must lie with the publisher, Charles C Thomas, who has recognized the need for such a work for many years and has made several attempts to have someone write on the subject. Upon seeing some material that the present editor was about to publish on the subject, Mr. Thomas thought it was what he had been looking for and suggested that I write the book he had in mind.

Certainly that was a flattering offer, but I knew all too well that the work in this field had become so specialized that any attempt I should make alone would be only a cheap imitation of what was needed. The present work is a result of a counter-proposal, namely, that leaders in each specialty should be asked to write chapters along the line of their special interest, and that I was to edit the project and contribute what I could in the field I knew best. From this joint effort there has resulted a work that has exceeded our fondest expectation.

While several leading authorities, much to our regret, were too preoccupied to join in the task, others have taken over with little more than the loss of the name of the outstanding specialist. As a matter of fact, the majority of the chapters are written by the most active research men in their particular fields at the present time, and the result is generally more down to date than what

might have been prepared by the men who are more widely known As it is most of the chapters are written by the present leaders in the field whose names are second to none in the work each has undertaken

It should be pointed out also that some of the authors have been asked and graciously consented to write on subjects a little afield from their greatest interest and consequently most effective potential Nobody however can detect in the least any defects in the text because of these special assignments Some are equally adept in more than one field

In such a work it is only natural to have some incongruities and repetition but the overlapping has been surprisingly little This is perhaps due to proper selection of the chapter headings In some such as the Saprophytic Reservoirs of Histoplasmosis the Geographic Distribution and the Skin Reactions repetition may be expected As also with the Introduction and History

It is recognized that a subject that is so new as the many problems involved in histoplasmosis will require frequent revision to keep knowledge up to date This we shall endeavor to do if there is a demand For the present we must see how this first attempt will fill the need

Recognition of all who helped in this effort would be rather difficult since there were numerous small favors performed by many who would not expect nor merit any more than verbal recognition Of those who performed noteworthy contributions mention should be made of the many who prepared chapters in the many specialties Their names appear at the heads of the respective chapters Dr Charles A Brasher has been most cooperative in lending his time and facilities of the institution as well as his secretary Mrs Louvina Baker Along with them are included Mrs Mary H Wicks Miss Dorothy Kent Mrs Hildred Cox who helped in typing the manuscript and Mrs Lois L Kern for assistance in proofreading and preparing the index

Most important in helping to bring this work to fruition were the untiring efforts of my late wife Mrs Ethyle M Sweany Mention should also be made of the assistance in many ways given by

my colleagues Dr David F Cotelick Dr Fred C Coller and Mr James L. Jones

Last but not least in fact the moving force behind the whole work is that of the publisher Mr Charles C Thomas and his able assistants whose work as usual is superb in every way

H C.S

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HISTOPLASMOSIS

INTRODUCTION

Henry Edmund Meleney

The development of our knowledge of histoplasmosis is one of the most fascinating stories in the annals of American medicine. It is a story studded with coincidences and with examples of mental alertness, persistence, ingenuity and cooperation in scientific research. It brings great credit to modern medical and biological investigation in the United States, where practically all of the fundamental research on the subject has been conducted.

Discovered in the Panama Canal Zone in 1905 by Samuel Taylor Darling, a young American pathologist who was in search of cases of visceral leishmaniasis, it was established as a fungus disease in 1912 by a Brazilian, Henrique da Rocha Lima, in Hamburg through comparison with a similar equine parasite. It remained unrecognized in the United States until a footnote in the writer's (1) paper on the histopathology of kala-azar in the Chinese hamster in 1925 called it to the attention of an alert pathologist, Cecil J. Watson, and his parasitological colleague, William A. Riley, in Minnesota when they discovered similar parasites in the autopsy tissues of an endogenous case of splenomegaly.

After another interval of four years a second coincidence occurred when the writer, then at Vanderbilt University in Nashville, Tennessee, received from John F. Kessel (2) in Los Angeles a tissue section from an autopsy containing parasites which were suspected of being Leishmania, but which resembled Histoplasma. The section was shown to Edna H. Tompkins who was studying blood cells by supravital staining. This led, after another three years, to the recognition by Dodd and Tompkins of the first case identified before death, in an infant born in the Vanderbilt University hospital, and to the cultivation and description of the parasite by DeMonbreun. Following the publication of that work, fatal cases were recognized with increasing frequency, mainly in the central valley of the United States, but also in the middle Atlantic

rats and other burrowing animals in an endemic area in Virginia and also from rats and skunks in Georgia where a very small proportion of the population reacted to histoplasmin Their remarkable persistence and ingenuity in isolation of the fungus not only from animals but from soil and other sources has added greatly to our exact knowledge of its natural habitat This work has demonstrated that animals are not a reservoir for infection of man but that the soil is the direct source of all infections

The dual form of Histoplasma capsulatum parasitic in the yeast form and saprophytic in the mycelial form adds to the interest and complexity of the infection In the yeast form at human body temperature it is essentially an intracellular parasite multiplying like Leishmania mainly in reticulo endothelial cells and thus differing from other systemic fungi which multiply more often in giant cells or extracellularly However Histoplasma infection differs from kala azar in producing areas of necrosis in which the yeast cells are present in enormous numbers Although the yeast form readily reverts to the mycelial form in culture at room temperature it apparently is not a source of infection to other persons or animals The mycelial form although requiring moisture for its development produces spores which are apparently resistant to drying and which give rise to human and animal infection mainly by inhalation of dust

The recognition of subclinical infections led to the supposition that similar to coccidioidomycosis there were probably early cases of histoplasmosis with acute respiratory symptoms Lung involvement as a terminal episode was first described by the writer (7) in a case of ascending renal infection but the first recognized group of cases of primary lung involvement was reported by Loosli and co workers in members of a farm family who had cleaned out a silo These cases recovered demonstrating that clinical infections are not always fatal The episode also emphasized the possibility of focal epidemics

The recognition of epidemics of acute pulmonary histoplasmosis is one of the most interesting developments in our knowledge of the disease Several were recognized retrospectively by finding miliary calcification in the lungs or by the association of the cases

region and in South America By 1910 the writer (3) was able to publish records of 32 cases and by 1945 Parsons and Zarafonetis summarized 71 cases

Another coincidence occurred with the appointment of Dr Amos Christie to the professorship of Pediatrics at Vanderbilt University With his knowledge of the epidemiology and pathology of coccidioidomycosis in California and on the advice of his former colleague Charles E Smith he and Peterson demonstrated a high prevalence of skin sensitivity to histoplasmin in his clinic patients thus suggesting the presence of subclinical infections in that area

Still another coincidence was the existence in the adjacent (Williamson) county of a long term study of the epidemiology of tuberculosis supervised by the Tennessee State Health Department under a grant from the Rockefeller Foundation Individual and family records of practically the entire population had been accumulated and showed the baffling fact that many individuals had calcification in the lungs with negative tuberculin reactions (4) Histoplasmin skin tests showed an exceedingly high prevalence of sensitivity and led to epidemiological studies which localized Histoplasma infections more in damp than in dry environments (5) and pointed to the chicken coop as the most frequent source of the fungus (6)

Meanwhile the problem of lung calcifications with negative reactions to tuberculin particularly in the midwestern states led to the application of the histoplasmin skin test first to student nurses by Palmer and then to population groups by Furcolow and others and thus to the mapping of the distribution of histoplasmin sensitivity in the United States The establishment of a histoplasmosis research center by the Public Health Service in Kansas City Kansas in a region of high prevalence under the direction of Michael L Furcolow made possible intensive studies along clinical epidemiological and immunological lines

After DeMonbreun s cultivation of Histoplasma capsulatum the fungus was frequently cultivated from human cases and DeMonbreun soon isolated it from a dog in Nashville This raised the question of possible reservoir hosts Emmons and his co workers pursued this possibility and recovered the fungus from dogs cats

tive Christie's use of ethyl vanillate at first gave promise but has proved ineffective in some cases and has a narrow therapeutic index The tendency for spontaneous cure of early infections and even of some disseminated cases makes it difficult to assess the effectiveness of therapeutic agents It may be that a non specific stimulation of resistance by a number of therapeutic agents can tip the balance in favor of the patient in a disease of low toxicity However with the development of amphotericin B an antifungal agent has been found which if administered intravenously appears to be curative in severe disseminated and chronic progressive pulmonary histoplasmosis and appears to shorten the course of early acute pulmonary cases The therapeutic conquest of the disease appears to be in sight

The fact that Histoplasma sometimes does not stain well in tissue sections with the usual hematoxylin stain may be the reason why it was overlooked for so many years by pathologists in the United States Even after it became recognized and was found in profusion in certain organs such as the lungs or the adrenal glands it sometimes could not be recognized in tubercle like lesions in other organs of the same case It also seemed to be absent from the caseous center of some larger lesions in which it was plentiful in macrophages at the periphery This difficulty has been overcome by the application of the Fuelgen reaction and the periodic Schiff stain to tissue sections bringing out in magenta color the polysaccharide capsule of the fungus even in apparently healed lesions Even a single organism can sometimes be identified in this way This advance is another aid in determining the presence of the infection in both man and animals

One of the interesting features of disseminated cases of histoplasmosis is the affinity of the fungus for the adrenal glands which often become large and caseous and have occasionally been the principal or only demonstrated focus of infection Another interesting localization is on the heart valves principally the aortic perhaps developing on an already scarred valve The striking feature of these lesions is that this is one location where the yeast form of Histoplasma multiplies extracellularly in the fibrin comprising the vegetation This makes such lesions similar in appearance to those

with the tearing down of buildings in which pigeons had nested The re-examination of the recovered patients by x-ray and serological and skin tests established the diagnosis and the cultivation of Histoplasma from the environment added conviction to the evidence The foresight of the Army and Public Health officials who originally studied some of these epidemics in preserving sera from patients which could be tested for Histoplasma antibodies illustrates the thoroughness with which search was made by those agencies for the etiologic diagnosis of the epidemics In 1957 Lehan and Furcolow recorded 38 known epidemics in the United States three in South Africa and one each in Peru and Venezuela Doubtless the list will continue to grow rapidly

Intensive work has been done in the past decade on the serological and immunological aspects of histoplasmosis The development of the skin test with histoplasmin an extract of the mycelial phase has been followed by three types of serological test namely complement fixation collodion particle agglutination and precipitation The permanence of the skin reaction suggests the possibility that the fungus remains antigenically active in the body for many years once it has gained entrance to the tissues The agglutination and precipitin reactions are apparently related primarily to the early acute stages of the disease whereas the complement fixation reaction is of longer duration The best types of antigen for these tests are still matters of study

Immunity in histoplasmosis is difficult to assess but there is evidence that it occurs It has been noted that epidemics have usually occurred either in areas outside the known highly endemic regions or among persons not native to those areas such as military personnel Animals can be protected by sublethal infections from heavy challenge inoculations

The specific therapy of disseminated histoplasmosis has until recently been a story of alternating hope and disappointment Pentavalent organic antimony compounds so effective in kala azar have been associated with temporary improvement in some cases but have failed in others Stilbamidine also effective in kala azar failed in the few cases in which it was tried and 2 hydroxystilbamidine which is curative in blastomycosis also proved to be ineffec

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caused by Candida which is not characteristically an intracellular organism in systemic infections This was brought out in a confusing case which occurred in an army hospital during World War II in which Candida was isolated in culture from the blood stream antemortem and from the aortic valve lesion and other organs at autopsy whereas histological sections showed extracellular yeast forms in the heart valves and intracellular forms typical of Histoplasma in the kidney and larynx (8)

The mass of epidemiological evidence which has been accumulated particularly by Furcolow and his associates indicates that man can become infected with the spores of Histoplasma from the soil not only in rural areas and especially in moist spots like chicken coops but also in urban areas such as Kansas City Here the older parts of the city show higher prevalence but even in the newer parts infection may come from the dust blown in from the heavily seeded rural areas Increase of infections has followed tornadoes and it is probably more than a coincidence that the highly endemic portion of the middle west is also the region most troubled by tornadoes That the spores escape into the air of laboratories where histoplasmosis research is conducted has also been demonstrated by the conversion of negative to positive skin tests in most of the personnel of the Public Health Service laboratory in Kansas City

These facts indicate that the prevention of human infection with Histoplasma capsulatum in endemic areas is practically impossible The spots seeded with the multiplying fungus in moist soil are usually sharply localized and there is no way to tell where they are Inhabitants of these areas can be warned to avoid inhalation of dust by wetting it down or if that is impossible by wearing a mask Infants should be especially protected from dust because the infection is more often acutely fatal at that age The inapparent infection caused by the inhalation of a few spores is usually a safe method of immunization and the acute respiratory infection usually terminates in healing and immunity The circumstances leading to chronic progressive pulmonary infection with cavitation and to visceral dissemination are not well understood but they are probably similar to parallel conditions in tuberculosis With the discovery of effective therapeutic agents and with the surgical resection

of resistant lesions, fatalities from the infection should be practically eliminated if the infection is recognized in time. The development of an effective vaccine for laboratory personnel and for certain population groups with known risk of exposure will doubtless receive greater attention in the future.

The problem of differentiation of histoplasmosis from pulmonary tuberculosis has been present ever since the recognition of the first few cases and the unexplained occurrence of pulmonary calcification with negative tuberculin reactions. The finding of both fungi and acid fast bacilli in the same lungs has occurred not infrequently, but many patients have been held for years in tuberculosis hospitals when they were actually suffering from histoplasmosis. With the rapidly spreading knowledge of this fact, such errors should soon disappear from areas where histoplasmosis is understood by chest physicians.

A review by the World Health Organization in 1952 recorded cases of histoplasmosis from Latin America (Mexico to Argentina), Europe, North and South Africa, the East Indies and Australia. There is no doubt of its endemicity in Latin America or South Africa, but skin test surveys in Europe have been almost uniformly negative and little is known about the prevalence farther east. The cases from the Philippines and Java may have been caused by a closely related fungus, of which Histoplasma farciminosus, which is prevalent in equines, seems to be the most likely. Future studies will undoubtedly elucidate this situation. It is of interest that early acute pulmonary cases have recently been recognized in Panama and that the Army and the Public Health Service have started an intensive epidemiological study there. It is also of interest that the first clinical cases were recognized recently in Costa Rica and Puerto Rico.

The writer has been a fortunate observer of this fascinating infection since its "resurrection" from obscurity rather than a contributor to its elucidation. He has attempted in this introduction to set the stage for what is presented in this volume by the resourceful and persistent workers whom the Editor has recruited as contributors. He has had the opportunity to review some of the chapters and has borrowed from them and from the literature freely

without listing references which are available in later chapters Students of medical history of mycology of immunology of epidemiology of pathology of clinical medicine and of preventive medicine will profit by the reading of this volume and the volume will undoubtedly stimulate the solution of some of the still unsolved problems of histoplasmosis

This introduction would be incomplete if it did not pay tribute to the man who first found the disease and described the causative organism Samuel Taylor Darling was born in Harrison New Jersey on April 6 1872 of English parentage After attending schools in Pawtucket Rhode Island he became a druggist He entered the College of Physicians and Surgeons of Baltimore and received his medical degree in 1903 at the head of his class After two years as instructor in histology and pathology at his alma mater and as a resident pathologist at the Baltimore City Hospital he was appointed in 1905 intern and physician at the Ancon Hospital in the Panama Canal Zone He also acted as pathologist and in 1906 became chief of laboratories of the Isthmian Canal Commission His alert mind set him searching for unfamiliar diseases at the autopsy table and it was in December 1905 that he encountered his first case of histoplasmosis He was in search for Leishmania and perhaps for that reason made smears from visible lesions and from spleen and bone marrow and applied a blood stain which immediately disclosed the parasites in good condition to study their morphology prior to the examination of fixed tissue sections It was undoubtedly his lack of familiarity with fungi which prevented him from making his own correction of his error in classifying the parasite as a protozoon

After his three cases of histoplasmosis encountered within a year no others were detected but his keen observations led to the discovery of two human cases of sarcocystosis Trypanosoma hippicum in horses and its transmission by sexual contact cutaneous leishmaniasis relapsing fever and the wide range of helminth and intestinal protozoan infections present in that region Yellow fever was coming under control but malaria was rampant and became one of his important interests

In 1913 and 1914 he accompanied Gorgas on a mission to South

Africa to investigate the cause of the high mortality among workers in the diamond mines. In 1915 he was appointed to the staff of the International Health Board of the Rockefeller Foundation and for the next three years served as head of a commission to study the causes of anemia in the people of Malaya, Java and Fiji. This work revealed the importance of hookworm as the cause of anemia, the value of worm counts in estimating intensity of infection, and the practical value of mass treatment without prior stool examination in heavily infected population groups. This and later work also led to his calling attention to the relationship between the geographical distribution of the two species of human hookworms and the ethnic origin and migration of their hosts.

From 1918 to 1920 Darling served as professor of Hygiene and director of the laboratories of Hygiene in the Medical School of Sao Paulo, Brazil, which the Rockefeller Foundation was assisting. Here he established a well equipped laboratory for teaching and research and carried out extensive studies on hookworm disease.

In 1920 he was forced by illness to return to the United States and in 1921 he became a fellow by courtesy in the department of medical zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

In 1922 Darling established for the Rockefeller Foundation a field laboratory at Leesburg, Georgia, for the investigation of malaria. Many young men were sent to him for field and laboratory training before proceeding to foreign countries as directors of malaria control campaigns. He also continued his research, developing the spleen index in children as a tool for measuring malaria endemicity, and identifying the larvae and breeding places of the three common species of Anopheles mosquitoes in the southeastern United States, so as to concentrate on the control of the breeding places of A. quadrimaculatus, the malaria transmitting species.

It was here that the writer had the privilege of spending a week with Darling and his latest pupil, Paul F. Russell, in October 1924. Russell had nearly completed his work on the differentiation of the larvae of A. quadrimaculatus, A. punctipennis and A. crucians. The days were spent in the field and laboratory, or conferring with famous visitors. The evenings were spent on the porch of the little

hotel with Darling reminiscing about his experiences especially during the pioneer days in the Canal Zone

On March 21 1925 Darling left the United States as a corresponding member of the malaria Commission of the Health Organization of the League of Nations to join in a malaria survey of Syria which then included Lebanon He was killed with two other *members of the party in an automobile accident on a mountain* road several miles from Beirut The Government of Lebanon erected a joint monument to the three victims at the sight of the accident which overlooks the Mediterranean Sea and the cape of land on which Beirut is located The writer had the privilege of paying homage to Darling at this monument in 1949

Darling was recognized during his lifetime as a world figure in tropical medicine He was the author of over 70 papers during his twenty years of research He received the honorary degree of Doctor of Science from the University of Maryland in 1923 He was president of the American Society of Tropical Medicine 1924 25 vice president of the American Society of Parasitologists 1925 and an honorary fellow of the Royal Society of Tropical Medicine and Hygiene London The Lebanese government awarded him the Medal of Merit posthumously

Darling s professional and personal qualities are best described in the obituary note in the annual report of the International Health Board of the Rockefeller Foundation for 1925

Dr Darling possessed in eminent degree the qualities of the successful investigator—joy in the exploration of nature and the search for truth command of methods and ingenuity in technique knowledge of the literature of his subject the scientific imagination and analytic type of mind His own example his attractive personal qualities and his generosity secured the enthusiastic loyalty and devotion of his assistants and fellow workers (9)

REFERENCES

1 Meleney H F The histopathology of Kala azar in the hamster monkey and man *Am J Path 1* 147 168 19[illegible]

2 Crumrine R M and Kessel J F Histoplasmosis (Darling) without splenomegaly *Am J Trop Med 11* 135 149 1931

3 Meleney H E. Histoplasmosis (reticulo-endothelial cytomycosis). A review. *Am J Trop Med* 20 603 616 1940

4 Cass R S Harrison F F Fuller R F Stewart H C and Williams W C. Pulmonary calcification and tuberculin sensitivity among children in Williamson County Tennessee. *Am Rev Tuberc* 47 379 387 1943

5 Zeidberg L D Dillon A and Cass R S. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County Tennessee. *Am J Pub Health* 41 80 89 1951

6 Zeidberg L D Ajello L Dillon A and Runyon L C. Isolation of *Histoplasma capsulatum* from soil. *Am J Pub Health* 42 930 935 1952

7 Meleney H E. Pulmonary histoplasmosis: report of two cases. *Am Rev Tuberc* 44 240 247 1941

8 Meleney H E. Problems in the histological diagnosis of histoplasmosis. *Proceedings of the Conference on Histoplasmosis 1952* Pub Health Mon No 39 1956 pp 11 16

9 The writer composed the biographical sketch of Dr Darling from the following obituary notices

Science 62 323 July 10 1925 by R W *Hegner*

Am J Trop Med 5 319 321 September 1925

J Parasitol 12 117 119 March 1926 by R W Hegner

12th Ann Rep (1925) Internat Health Board Rockefeller Foundation 1926 p 108

THE HISTORY OF HISTOPLASMOSIS

GERALD L. BAUM

In the course of the vast amount of work that has been done in the last half century in the medical sciences it has become necessary for the men who devote their efforts to this study to concentrate on the diseases that are relatively common. This is simply explained by the urgency of the work and the painful limitation of time.

In the instance of histoplasmosis for many years it was felt that the number of men afflicted with the disease was very small and consequently the work done was little. To the research man the disease was a curiosity caused by a rarely seen organism; to the clinician the whole situation was of little interest since the number of patients was miniscule and the treatment non existent.

With abrupt suddenness the story changed pace. From the early 1940's until the present there has occurred an unbelievably rapid accumulation of knowledge about histoplasmosis since it was realized that instead of being the rare disease it was originally thought to be histoplasmosis afflicts millions of men (and women) in the endemic area and quite a few outside of it.

This work has been accomplished through the efforts of many dedicated and intelligent men who had the perception to see what at first were obscure associations and who had the courage to speak out when they were sure of themselves even in the face of well established opinions to the contrary. This is not unusual among physicians but this has happened with such dramatic speed in the case of histoplasmosis that the attention of the whole profession has been drawn to it.

In 1905 Dr. Samuel Taylor Darling went to the Ancon Canal Zone hospital as pathologist. He was 33 years old but only two years out of medical school and had qualified by examination for the appointment. Soon after arriving he was asked to perform an

Figures 4 [illegible] and 11 of this chapter are reprinted with permission of the publishers of the *New England Journal of Medicine* ([illegible])



Fig. 1. Samuel Taylor Darling, taken about 1910

autopsy on a negro from Martinique who had died from an overwhelming infection. The examination revealed gross lesions resembling disseminated tuberculosis but the microscopic study done so carefully by Dr. Darling yielded rather startling findings. Intracellular organisms seen in *histiocytes* resembled *Plasmodia* and in addition seemed to have a *capsule* (11).

These intracellular organisms aroused the interest of Dr. Darling because he had been directing his studies toward the finding of an American form of Leishmania. He had first thought that these intracellular organisms represented Leishmania but on close inspec-

tion he noted that no kinetoplast was visible He therefore felt that what he had seen represented a new organism which he named Histoplasma capsulatum (14)

Dr Darling s observations prompted him to classify his Histoplasma as an animal parasite probably because of its close resemb



Fig 2 Drawings of the organism Histoplasma capsulatum as they appeared to Darling This plate appeared in a summary article written by Darling about the three cases of histoplasmosis that he had observed Reprinted with permission of the publishers of the *Archives of Internal Medicine* (2 107 193 1908)

lance to Leishmania It is of interest to note that of the many observations made of the organism by Dr Darling this represented one of the few errors

One cannot reflect on the work of Dr Darling and of the accuracy of his observations as manifested by drawings which appeared in subsequent publications (16) without being amazed The work was carried on under almost primitive conditions at least by modern standards Certainly the steaming jungles of Panama did not provide a stimulating atmosphere for hard work In addition the microscopes of the day were not ones which revealed their secrets easily The work done on histoplasmosis by Dr Darling is one of the truly fine contributions by an American research physician

The original case observed by Dr Darling was seen in 1905 The following year Dr Darling observed two more cases (15) The second being in a Martinique Negro and the third case being in a Chinese laborer who had been in Panama for 15 years prior to his illness All three cases reported by Darling were adults all were disseminated and all cases died

Though it was Darling whose first report has gained recognition one must mention the work of Richard P Strong who in 1906 reported organisms which he observed while working in the Philippine Islands which were consistent with H capsulatum (66) His report preceded Dr Darling s however he later expressed the belief that the organism which he saw and described was H farciminosum By comparison Darling s description was considerably more complete and his drawings more correct therefore he has been given the prime credit The present author feels unqualified to decide who should receive the prior credit but it is apparent that Darling s work was the more complete and therefore deserves mention at least for that fact

Following Darling s work and the publication which resulted from his observations nothing was seen of histoplasmosis until shortly prior to World War I Henrique da Rocha Lima a Brazilian who spent 19 years in The Institute of Tropical Diseases in Hamburg Germany reported in 1912 the following observation A comparison of the microorganism of Darling with Leishmania and

tion he noted that no kinetoplast was visible He therefore felt that what he had seen represented a new organism which he named Histoplasma capsulatum (14)

Dr Darling s observations prompted him to classify his Histo plasma as an animal parasite probably because of its close resemb

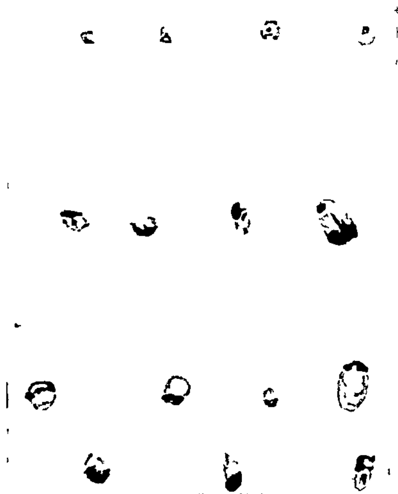


Fig 2 Drawings of the organism Histoplasma capsulatum as they appeared to Darling This plate appeared in a summary article written by Darling about the three cases of histoplasmosis that he had observed Reprinted with permission of the publishers of the *Archives of Internal Medicine* (? 107 123 1908)

Dr Henry E Meleney who showed him sections of visceral leishmaniasis in Chinese hamsters Dr Darling called attention to the similarity of this infection to histoplasmosis and demonstrated sections from his cases This seemingly casual meeting was an opportune one as subsequent events were to prove

In 1926 Drs William Riley and Cecil J Watson described a case of histoplasmosis originating in a resident of Minnesota (56) This was the first case well documented since Darling s three were reported and the first case recognized in the United States A case seen in Europe had been described as histoplasmosis by Riehl in 1924 25 (55) but the documentation noted was not convincing

The case described by Riley and Watson was a woman with disseminated disease who at autopsy demonstrated widespread involvement The findings were identical with Darling s cases It is of interest to note that Riley and Watson speculated on the occurrence of more cases in the U S in the following way As we attempted to point out the finding of an endemic case of histoplasmosis in Minnesota is evidence that it is not a purely tropical disease but that it must be considered as a possible factor in any obscure case of splenomegaly accompanied by irregular pyrexia emaciation and anemia (57)

In 1929 Dr Meleney then at Vanderbilt University School of Medicine received from Dr John F Kessel of Los Angeles tissue sections from a local autopsy showing Histoplasma like organisms Dr Meleney showed these to Dr Edna H Tompkins who was studying supravital staining of blood cells This casual exchange of information was to prove significant In 1932 Dr Katherine Dodd was caring for a child on the Pediatric service at Vanderbilt who was suffering from an unknown type of anemia (19) Dr Dodd was aware of Dr Tompkins interest in supravital staining of cells and she asked Dr Tompkins to use this technique on her patient What subsequently happened is best quoted from Dr Tompkins Once detected supravitally by virtue of intense refractivity and droplets of dye they (Histoplasma capsulatum) could be demonstrated in fixed films in which they had been overlooked previously Dr Meleney once showed me one of these sections (of a case of histoplasmosis) just because of my interest in the reticulo endothelial

with yeast showed a much greater similarity of their quality with yeast than with protozoa (13) This observation had been made by da Rocha Lima after comparing Darling s material with tissue and smears from cases of epizootic lymphangitis caused by H farcimi nosum and Leishmania This observation which was the result of exacting work has proven in subsequent years to be correct and certainly represents a significant contribution For completeness it should be mentioned that da Rocha Lima also pointed out that Darling s observation of flagellate cell forms of H capsulatum was probably an erroneous one

In October 1924 Dr Darling who was then in charge of the Malaria Experiment Station at Leesburg Georgia was visited by



Fig 3 Henrique da Rocha Lima



Fig. 2 William A. DeMonbreun

Dr William A DeMonbreun pathologist at Vanderbilt was notified of the diagnosis made by Dr Dodd and Dr Tompkins The child's demise was imminent and Dr DeMonbreun was prepared when it occurred to make cultures on a variety of media These proved successful and H capsulatum was grown Dr DeMonbreun after culturing the organism worked out the details of its morphology culture and growth characteristics (17) His work was of such excellence that little has had to be added up to the present time His studies included injecting animals and recovering the organism from the blood during life and again after the animals died He established beyond any doubt the etiologic relationship between the



Fig 1 Katherine Dodd

system in general That picture came back to me as I puzzled about the parasites that filled the circulating monocytes of the infant under study (61) According to Dr Tompkins Dr Meleney was in the building at the time she made her observation She called him and he came running down the stairs took a short look through the microscope and uttered enthusiastic corroboration of Dr Tompkins suspicions (68) Dr Ernest Goodpasture subsequently agreed with the impressions of both Dr Tompkins and Dr Meleney



Fig [illegible] William A DeMonbreun

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organism which he had cultured Histoplasma capsulatum and the disease histoplasmosis in the experimental animals He established the dimorphism of the organism and he described and photo graphed the tuberculate spores which since that time have been recognized as diagnostic of the mold phase of the organism In addition DeMonbreun concluded that the saprophytic form of H capsulatum probably existed free in nature As can be seen the micro biological characteristics of the organism were brilliantly worked out by Dr DeMonbreun His only failure was in trying to change the name of the disease to cytomycosis Despite the fact that Dr Meleney was a strong proponent of this name it never caught on (47) DeMonbreun was only 34 years old at the time he did his work and the resulting paper which was presented in November of 1933 at the 29th annual meeting of the American Society of Tropi cal Medicine was a masterpiece (17)

In 1933 at a meeting of the American Association of Patholo gists and Bacteriologists a few months before DeMonbreun had presented his work Dr G H Hansmann and Dr John R Schenken presented work related to the isolation of a fungus which they considered to be Sepedonium (35) Review of their work revealed that this was undoubtedly the first culture of H capsulatum Their report preceded Dr DeMonbreun s by approximately six months however it lacked the completeness of Dr DeMonbreun s and has been subsequently overshadowed However as with Dr Strong and Dr Darling one is faced with the decision of whom should receive prior credit for the cultural work Hansmann and Schenken most certainly preceded DeMonbreun in their report but DeMon breun s work was considerably more complete In all justice it would seem that the word of Schenken who has approved of the statement of Conant DeMonbreun Hansmann and Schenken finally cultured the organism and proved it to be a fungus should be taken (61)

One cannot but wonder at the rather amazing coincidence that both the original identification of the organism and the disease as well as the first culture of the organism should be accomplished almost simultaneously in two separate laboratories

Despite the excellence of work done up to this point histoplas

mosis was still considered to be a disease of little significance in the United States. It was felt to be a rare generalized infection involving the reticulo-endothelial system which was uniformly fatal (17).

Bernard *et al.* reported in 1931 (2) 327 of 1,000 New York city adolescents tested were negative to tuberculin. Of these 11 (3.4%) showed fibrous and/or calcific scars in the lungs and/or hili. In 1933 Crabtree *et al.* (11) made the observation that in Sullivan county, Tennessee, over 1/5 of persons older than 5 years of age with pulmonary calcifications were negative to tuberculin. In 1938 both Nelson *et al.* and Gass *et al.* (30, 19) noted that about 40% of tuberculin-negative children and young adults had pulmonary calcifications on x-ray. In 1938 Dr. Michael L. Furcolow and others participated in a conference in Hagerstown, Md., at which general agreement was reached that these above-mentioned observations did in fact represent a true relationship between negative reactions to tuberculin and calcification in the lungs (29).

In 1939 Crimm and Short (12) reported coincidence of pulmonary calcifications and negative tuberculin reactions in Evansville, Indiana. The same year Lumsden (15) on the basis of prominent coincidence of negative tuberculin reactions and pulmonary calcifications in Giles county, Tennessee, where the tuberculosis mortality rate was high, questioned the value of the tuberculin test as an index of tuberculous infection.

In 1943 there appeared a report of observations of a large number of chest x-rays made on men being inducted into the service (17). Because the number of calcifications present on chest x-ray could cause rejection from military service, these concretions were noted rather carefully. Review of these films yielded the interesting consistent observation that calcifications of the lung were much more frequently seen in people who came from the Mississippi-Ohio River Valley area.

In 1941 a case of laryngeal histoplasmosis was reported by VanPernis *et al.* (69) and what makes this of particular interest is that the authors used a filtrate of a broth mycelial phase culture of the organism isolated from the case to skin test the patient. Delayed positive reactions were noted to both undiluted and several ten-fold dilutions of the material. This represented the first application

of histoplasmin as a skin test antigen in man At about the same time use of the filtrate and a heat killed yeast vaccine as a skin antigen in animals was found effective (70)

The next crucial event was the appointment in 1943 of Dr Amos Christie as professor of pediatrics at Vanderbilt University Dr Christie had been working with Dr Charles F Smith prior to coming to Vanderbilt and it was through this association that he had witnessed the elucidation of the problem of coccidioidomycosis Shortly after his arrival at Vanderbilt he was puzzled by the presence of pulmonary calcifications in persons with negative tuberculin tests He consulted Dr Smith about this observation and he sug



Fig 6 Amos Christie

gested that histoplasmosis might be responsible for the calcifications in the chests of people who were negative to both tuberculin and coccidioidin (61) It is rather prophetic that in a letter dated Dec 30 1913 Dr Smith should make the following comment P S Old Histoplasma holds a soft spot probably because I know so little about it but DeMonbreun did do his epochal transmission studies at Vanderbilt and the status of histoplasmosis now is like that of coccidioidal granuloma before Gifford and Dickson showed mild infections occurred (61)

The hint had been given previously by Dr DeMonbreun because in 1939 he had said It is probable then that the disease is more common than is generally supposed possibly because the disease may occur in a relatively mild and non fatal form and not be recognized (18)

It must be remembered that at this point Parsons and Zarafonetis (52) were preparing their classic report and review in which 71 cases of histoplasmosis were collected from 1905 to the time of writing All of these were fatal cases and it was their opinion that this was the rule Their work was detailed and represents the last time that histoplasmosis was referred to as a rare uniformly fatal disease

During 1944 Christie and Dr J C Peterson tested approximately 180 children internes and residents with skin test antigens They found a high incidence of positive reactions to histoplasmin in the face of negative tuberculin tests (9) This then was the big lead from which the epidemiology of histoplasmosis has been worked out

Dr Christie communicated his information to Dr Edwards A Park professor of pediatrics at Johns Hopkins (61) who suggested to Christie that he discuss the matter with Dr Carroll Palmer an officer of the U S Public Health Service It was Dr Palmer s study of the skin sensitivity in student nurses which already had revealed marked geographic variations in the prevalence of pulmonary calcifications in tuberculin non reactors Dr Palmer had suspected that spores of saprophytes were the cause of the non tuberculous pulmonary calcifications Dr Chester W Emmons expressed the feeling that Histoplasma capsulatum was the cause (61) Dr Palmer and

Dr Michael L Furcolow visited Dr Christie in March of 1945 They were apparently fascinated with his results Dr Palmer then obtained histoplasmin from Dr Emmons and incorporated histoplasmin skin testing in his nationwide project of skin testing student nurses In May of 1945 Dr Palmer reported the results of his work which showed enormous differences in the skin sensitivity to histoplasmin between persons living in different areas of the U S (50) It was then that Dr Christie s original observation was corroborated without question There was a high degree of correlation between positive histoplasmin skin tests and pulmonary calcifications in people reacting negatively to tuberculin

Dr Palmer s studies were done on several thousand subjects and required close cooperation of several people Dr Charlotte Silverman and Dr Caroline Chandler did the actual skin testing and Dr Henry Zwerling reviewed all of the x rays

And so it was that with the reports from both Christie and Palmer the existence of a benign subclinical form of histoplasmosis was established Almost all of the cases tested were without symptoms and have remained completely well A few cases were reported by Christie to have shown the organism in the tissues (8) The outgrowth of Dr Palmer s work which involved a large number of persons from many different areas was the mapping out of the geographical incidence of the infection (51) The original work has been confirmed on numerous occasions subsequently

A more recent contribution to the epidemiology of histoplasmosis was made by Dr P Edwards who mapped the worldwide incidence of histoplasmin sensitivity These papers established areas of endemicity in previously unsuspected regions of the world (22 23 48)

Before carrying the story further it seems fitting to pause and reflect on the significant place in this drama that is held by Vanderbilt University Dr Meleney was there with the crucial information which prompted Dr Dodd Dr Tompkins and Dr DeMonbreun to do their original work on the isolation and identification of Histoplasma capsulatum The culture of Histoplasma originally grown by Dr DeMonbreun served as the source of histoplasmin which was used by Dr Christie some 10 years later in his skin test survey which



Fig 7 Carroll E Palmer

established the existence of the benign form of histoplasmosis What makes this tale most fascinating indeed is that the significant contributors continue to work and are available for comment and elucidation

It must now become obvious that histoplasmosis had outgrown its status as a rare uniformly fatal disease of little concern to American physicians More and more people became interested in this disease and their combined efforts produced a rapid accumulation of facts

In 1947 Tenenberg developed the first of the serologic meth

ods of diagnosis the complement fixation test (67) This gave great promise of clarifying diagnostic problems since the experience with coccidioidomycosis had pointed to the pertinence of serologic findings Subsequent to the description of the complement fixation test two other techniques have been described a precipitin test (59) and a collodion agglutination test (60) These tests particularly the complement fixation test have been useful in helping to clarify epidemics acute primary infections and chronic cavitary infections

The important aspect of cross reactions of the serologic tests with other deep fungus diseases has been worked out in detail (6 7)

Despite the fact that the growth characteristics of H capsulatum were well known by the late 1940 s particularly that at room temperature and therefore in nature the organism must grow as a mold there was no absolute proof that the organism did exist in nature and therefore an important fact was missing from the pattern of pathogenesis of histoplasmosis All evidence accumulated up till this point indicated that the infection was primarily a respiratory one Final proof that the organism does exist in nature was contributed by Dr Chester W Emmons mycologist for the U S Public Health Service (26) It was in October 1948 that he cultured Histoplasma from two soil specimens the site being from a mound of earth at the entrance of a rat burrow under the edge of a chicken house The tuberculate spores which are diagnostic of the mold phase of Histoplasma capsulatum were actually microscopically demonstrated in the soil Positive cultures were obtained only after 156 specimens were negative pointing up the perseverence necessary for the successful completion of such a job

Following Dr Emmons original isolation of the organism from nature several people have cultured the organism from soil samples in various areas around the country A very significant contribution made by Dr Libero Ajello and Dr L D Zeidberg was that H capsulatum more heavily contaminates the soil around chicken houses than any other area (1) The relation of this fact to the discussion of epidemics will become apparent

In 1939 Dr DeMonbreun had demonstrated the first case of spontaneous histoplasmosis in a dog (18) Some ten years later Dr Emmons demonstrated spontaneous histoplasmosis in 10 species of

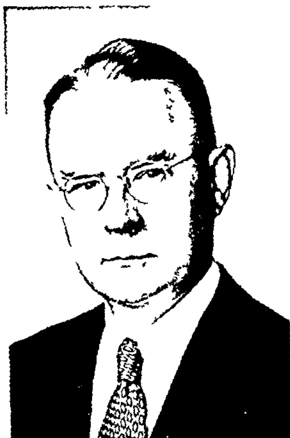


Fig. [illegible] Chester W. Emmons

animals including dogs and cats (21, 25, 27). He suggested that animal infection was a good index of the incidence of the disease in any given region (58).

In addition to his epidemiological work with Dr. Carroll Palmer, Dr. Michael L. Furcolow has contributed greatly to the elucidation of many problems concerning histoplasmosis. In 1945 Dr. Furcolow established in Kansas City, Kansas, a field station of the U. S. Public Health Service for the study of histoplasmosis. From this center has emanated considerable information. Dr. Furcolow demonstrated H. capsulatum in air samples (47). He was the



Fig 8 Michael L Furcolow

first to call attention to the chronic pulmonary cavitary form of histoplasmosis and to note its confusion with tuberculosis (32) In fact in a survey in a nearby sanatorium he uncovered a surprisingly large number of cases of histoplasmosis that had been called tuberculosis for many years Dr Furcolow has been active in epidemiological work ever since he and Dr Palmer did their original skin test studies Dr Furcolow and his colleagues have investigated many of the so-called epidemics of histoplasmosis and have analyzed them in great detail (28) He has had experience with a large number of clinical cases of histoplasmosis and has recently reported the use of new therapeutic tools (39)

The first of the epidemics occurred in soldiers at Camp Gruber Oklahoma (1 10) and since that time many interesting examples of these focal outbreaks of histoplasmosis have been reported (28) The association with soil around chicken houses and with bat and pigeon excreta has been striking Thirty epidemics have been reported involving some 350 persons The many similarities in these various epidemics have been pointed out by Furcolow and Grayston (28) The pattern is recurrent exposure to the spore containing dust mild to severe widespread pulmonary disease followed generally by a recovery and often calcification in the pulmonary lesions Very few deaths have been reported A rising serologic titer during the acute phase often has been demonstrable (12 13)

In 1953 Dr Thomas I Puckett examined specimens of so called tuberculomas removed from patients at the Fitzsimmons Army Hospital Making use of special fungus stains he demonstrated organisms which were morphologically compatible with Histoplasma capsulatum (53)

The genesis of histologic recognition of H capsulatum has depended in great part on development of special stains Bauer in 1933 (3) described the selective staining by Schiff s reagent of aldehyde groups released from carbohydrates by oxidation with chromic acid In 1946 McManus (46) reported good demonstration of mucin using a similar technique but replacing the chromic acid with periodic acid In 1946 Gomori (31) using the chromic acid oxidizing reaction replaced the Schiff s reagent with methenamine silver nitrate and obtained brilliant black staining of carbohydrates Lillie (40) compared the effects of Bauer s Feulgen s Gram s and Gram Weigert s stains on various parasites and mentioned the selectivity of the Bauer s stain for yeast cells among them Histoplasma Hotchkiss (36) in 1948 using the same technique described by McManus (46) mentioned the effective staining of chitin and demonstrated stained yeast cells by this method In 1951 the implications of these stains in diagnosing the presence of fungi in human tissues were crystallized by Kligman *et al* (38) The Hotchkiss McManus technique was considered superior to the Bauer s by these authors In addition they employed a counter stain to bring out the positive



Fig 10 Thomas F Puckett

structures By making use of both Bauer s and Hotchkiss McManus reagents and adding some original variations Gridley (33) in 1953 described a stain which proved superior to all others In 1955 Grocott (34) applied the technique described by Gomori (31) to fungi and produced a stain considered by many the most effective fungus stain yet developed

Dr Puckett used the periodic acid Schiff stain (Hotchkiss McManus) and stated his conclusion thusly after thorough study of a good many of the lesions I became convinced that they were due to histoplasmosis (61) These observations by Dr

Puckett are extremely important for they opened a new approach to the diagnosis of histoplasmosis. By using conventional stains, that is *hematoxylin and eosin*, the organism is often difficult, if not impossible, positively to identify, especially when dead. Since culture is not only tedious and time consuming but in healed lesions often negative, it is only by making use of special fungus stains that the etiology of such healed granulomatous lesions can be suspected.

In 1951 the final link in the chain of pathogenesis was forged by Dr. Manuel Straub. Dr. Straub came from Holland in that year with the intention of investigating the morphology of tuberculosis in the American Negro. His stop in Cincinnati, however, proved



Fig. 11. Manuel Straub

crucial and Dr Straub revised his plans and spent the rest of his time studying the morphology of histoplasmosis Dr Straub had had extensive experience in tuberculosis having received his training with Ghon in Prague In Cincinnati he investigated the lungs of more than 100 consecutive autopsies the only selection being that the specimens come from patients who had no active pulmonary disease In 67% of these lungs Dr Straub was able to identify a healed primary pulmonary lesion in which could be demonstrated the organism H capsulatum (65) The significance of this observation is obvious when one considers that it established the specificity of histoplasmin skin testing and proved beyond any question that histoplasmosis is in the overwhelming majority of cases a primary pulmonary disease

Working with Dr Straub was Dr Jan Schwarz whose interest and work in mycology have dated from 1950 Dr Schwarz had originally trained at the University of Prague with Ghon as did Dr Straub Dr Schwarz arrived in Cincinnati in 1947 and was soon impressed with the number of splenic calcifications he noted at autopsy This interest and curiosity prompted him to compare the incidence of splenic calcifications in Cincinnati with other parts of this country and the world and it soon became apparent that in Cincinnati the incidence of splenic calcifications was considerably higher than in the other areas checked (62) The organism H capsulatum was demonstrated in the splenic foci especially those which show concentric laminations of calcifications This contribution established the rather unusual fact that despite the benign character *of the primary infection in the overwhelming majority of people* it is accompanied regularly by bloodstream dissemination of the organism In collaboration with Dr Bernardo Serviansky Dr Schwarz demonstrated what had been suggested in the paper on splenic calcification that the radiologic picture of histoplasmic calcific pulmonary lesions had not only characteristic configuration but characteristic size (63)

In recent years many other interesting observations have been made such as the identification of a separate species of Histoplasma by Vanbreuseghem in Africa (Histoplasma duboisii) with its attendent large forms in human tissues (21) the demonstration that Ameri

can strains of H capsulatum can produce large forms in hamsters (20) the fractionation of antigenic substances from the yeast cell of Histoplasma capsulatum (5) the electron microscopic finding that the apparent capsule of Histoplasma capsulatum is no capsule at all but represents contraction of the organism within the tissues (54) and finally the development of chemotherapeutic agents which give great promise of being effective against histoplasmosis clinically (11 64)

Because of the rapid development of knowledge in the study of this disease much crucial and excellent work that has been done has not been mentioned here This is merely a reflection of the limitation of space and certainly no reflection of the relative importance of the work done These are the highlights Seldom in the history of the study of the ills of man has so much knowledge accumulated about any one malady so rapidly Several important problems remain to be solved among them vaccination explanation of geographic incidence etc but with the results of the past 15 years as inspiration there is no question but that Aristotle s admonition Time is a great babbler will be rapidly fulfilled

REFERENCES

1 Ajello L and Zeidberg L D Isolation of Histoplasma capsulatum and Allescheria boydii from soil *Science 113* 662 1951

2 Barnard M W Amberson J B and Loew M F Tuberculosis in adolescents a study of 1000 school children in New York City made under the auspices of the Bellevue Yorkville health demonstration in 1930 *Am Rev Tuberc 23* 93 641 1931

3 Bauer H Mikroskopisch chemischer Nachweiss von Glykogen und einigen anderen Polysacchariden *Ztschr mikroskop anat Forsch 33* 143 160 1933

4 Cain J C Devins E J and Downing J F An unusual pulmonary disease *Arch Int Med 79* 6 6 641 1947

5 Campbell C C Antigenic fractions of H capsulatum *Am J P h Health 43* 712 717 19 3

6 Campbell C C Cross reactions of mycotic antigens Pub Health Monogr No 39 19 6 pp 144 148

7 Campbell C C and Binkley C F Serology diagnosis with respect to histoplasmosis coccidioidomycosis and blastomycosis and the problem of cross reactions *J Lab & Clin Med 42* 896 906 1953

8 Christie A Histoplasmosis and pulmonary calcifications *Ann New York Acad Sc 50* 1 93 1 98 19 0

9 Christie A and Peterson J C Pulmonary calcification in negative reactors to tuberculin *Am J Pub Health* 35 1131 1147 1945

10 Commission on acute respiratory diseases in collaboration with W A Mickle Jr Studies on the causation of an unusual disease at Camp Gruber Okla *Arch Int Med* 80 203 204 1947

11 Crabtree J A Hickerson W D and Hickerson A P Tuberculosis studies in Tennessee a community study of the prevalence of tuberculosis in the Negro *Am Rev Tuberc* 8 suppl 6 1933

12 Crimm P D and Short D M Tuberculin anergy in cases with pulmonary calcifications *Am Rev Tuberc* 39 61 69 1939

13 da Rocha Lima H Beitrag zur Kenntnis der Blastomykosen Lymphangitis epizootica und Histoplasmosis *Zentralbl Bakt* 67 233 249 1912 1913

14 Darling S T Protozoon general infection producing pseudotubercles in lungs and focal necrosis in liver spleen and lymph nodes *J A M A* 46 1283 1285 1906

15 Darling S T Histoplasmosis fatal infectious disease resembling kala azar found among natives of tropical America *Arch Int Med* 2 107 123 1908

16 Darling S T Morphology of parasite (Histoplasma capsulatum) and lesions of histoplasmosis fatal disease of tropical America *J Exper Med* 11 515 531 1909

17 DeMonbreun W A Cultivation and cultural characteristics of Darling s Histoplasma capsulatum *Am J Trop Med* 14 93 125 1934

18 DeMonbreun W A Dog as natural host for Histoplasma capsulatum *Am J Trop Med* 19 565 87 1939

19 Dodd K and Tompkins F H Case of histoplasmosis of Darling in infant *Am J Trop Med* 14 127 137 1934

20 Drouhet E and Schwarz J Comparative studies with 18 strains of Histoplasma *J Lab & Clin Med* 47 128 139 1956

21 Dubois A Janssens P G Brutsaert P and Vanbreuseghem R Un cas d histoplasmose africaine avec une note mycologique sur H duboisii n sp *Ann Soc Belge de Med Trop* 32 569 585 1952

22 Edwards L O Ceser A C Kjobbye F H Meijer J and Christensen O W Histoplasmin testing in Africa and southern Asia *Am J Trop Med* 5 224 251 1956

23 Edwards P Q and Klaer J H World wide geographic distribution of histoplasmosis and histoplasmin sensitivity *Am J Trop Med* 5 235 257 1956

24 Emmons C W Histoplasmosis in animals *Tr New York Ac d Sc* 2 248 254 1949

25 Emmons C W Histoplasmosis animal reservoirs and other sources in nature of pathogenic fungus Histoplasma *Am J Pub Health* 40 436 440 1950

26 Emmons C W Morlan H B and Hill E L Isolation of Histoplasma capsulatum from soil *Pub Health Rep* 64 892 896 1949

27 Emmons C W Morlan H B and Hill E L Histoplasmosis in rats and skunks in Georgia *Pub Health Rep* 64 1423 1430 1949

28 Furcolow M L and Grayston J T Non tuberculous chest diseases occurrence of histoplasmosis in epidemics *Tr Nat Tuberc A* 48 83 91 19

29 Furcolow M L Personal communication

30 Ca s R S Cauld R L Harrison L F Stewart H C and Williams W C Tuberculosis studies in Tennessee and roentgenological evidence of tuberculous infection in relation to tuberculin sensitivity in school children *Am Rev Tuberc* 38 111 117 1938

31 Gomori C A new histochemical test for glycogen and mucin *Am J Clin Path Tech Bull* 10 177 1 9 1946

32 Grayston J T and Furcolow M L Occurrence of histoplasmosis in epidemics epidemiologic studies *Am J Pub Health* 43 66 6 6 19 3

33 Gridley M F A stain for fungi in tissue sections *Am J Clin Path* 23 303 307 19 3

34 Grocott R G A stain for fungi in tissue sections and smears using Gomori s methenamine silver nitrate technic *Am J Clin Path* 5 9 5 9 9 19 5

35 Hansmann C H and Schenken J R Unique infection in man caused by new yeast like organism pathogenic member of genus Sepedonium *Am J Path* 10 31 538 1934

36 Hotchkiss R D A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations *Arch Biochem* 16 131 141 1948

37 Ibach M J Larsh H W and Furcolow M L Isolation of H Capsulatum from air *Science* 119 71 19 4

38 Kligman A M Mescon H and DeLamater E D The Hotchkiss McManus stain for the histopathologic diagnosis of fungus disease *Am J Clin Path* 21 86 91 19 1

39 Lehan P H Yates J L Brasher C A Larsh H W and Furcolow M L Experiences with the therapy of sixty cases of deep mycotic infections *Dis Chest* 3 97 C14 1957

40 Lillie R D Reactions of various parasitic organisms in tissues to the Bauer Feulgen Gram and Gram Weigert methods *J Lab & Clin Med* 32 76 88 1947

41 Long E R and Stearns W H Physical examination at induction standards with respect to tuberculosis and their application as illustrated by review of 53 400 x ray films of men in army of United States *Radiology* 41 144 150 1943

42 Loosli C G Grayston J T Alexander E R and Tanzi F Epidemiological studies of pulmonary histoplasmosis in farm family *Am J Hyg* 55 39 401 1952

43 Loosli C G Procknow J J Tanzi F Grayston J T and Coombs L W Pulmonary histoplasmosis in farm family three year follow up *J Lab & Clin Med* 43 669 69 19 4

44 Louria D B Feder N and Emmons C W Amphotericin B in experimental histoplasmosis and cryptococcosis *Antibiotics Annual 1956 1957* New York Medical Encyclopedia Inc pp 870 877

45 Lumsden L L Dearing W L and Brown R A Questionable value of skin

testing as a means of establishing an epidemiological index of tuberculous infection *Am J Pub Health* 29 ... 31 1939

46 McManus J F A Histological demonstration of mucin after periodic acid *Nature* 158 202 1946

47 Meleney H E Histoplasmosis (reticulo endothelial cytomycosis) a review with mention of 13 unpublished cases. *Am J Trop Med* 20 603 616 1940

48 Mochi A and Edwards P Q Geographical distribution of histoplasmosis and histoplasmin sensitivity *Bull World Health Org* 5 229 259 1952

49 Nelson W E Mitchell A C and Brown E W The intracutaneous tuberculin reaction associated with calcified intrathoracic lesions *Am Rev Tuberc* 37 311 314 1938

50 Palmer C E Non tuberculous pulmonary calcification and sensitivity to histoplasmin *Pub Health Rep* 60 513 520 1945

51 Palmer C E Geographic differences in sensitivity to histoplasmin among student nurses *Pub Health Rep* 61 4 5-481 1946

52 Parsons R J and Zarafonetis C J D Histoplasmosis in man report of seven cases and a review of seventy-one cases *Arch Int Med* 75 1 23 1945

53 Puckett T F Pulmonary histoplasmosis study of 21 cases with identification of H capsulatum in resected lesions *Am Rev Tuberc* 67 453 476 1953

54 Ribi E and Salvin S B Antigens from the yeast phase of Histoplasma capsulatum I Morphology of the cell as revealed by the electron microscope *Exper Cell Research* 10 394 401 1956

55 Riehl G Durch pathogene sprosspilze bedingte granuloma *Arch Dermat u Syph* (Berl) 148 39? 398 1924 1925

56 Riley W A and Watson C J Histoplasmosis of Darling with report of a case originating in Minnesota *Am J Trop Med* 6 271 282 1926

57 Riley W A and Watson C J Darling s histoplasmosis in the United States the possibility of the further occurrence of cases *Minnesota Med* 9 97 1926

58 Rowley D A Haberman R T and Emmons C W Histoplasmosis pathologic studies of fifty dogs and fifty cats from Loudoun County Virginia *J Infect Dis* 95 98 108 1954

59 Salvin S B and Hottle G A Serologic studies on antigens from Histoplasma capsulatum Darling *J Immunol* 60 57 66 1948

60 Saslaw S and Campbell C C A method for demonstrating antibodies in rabbit sera against histoplasmin by collodion agglutination technique *Proc Soc Exper Biol & Med* 68 559 562 1948

61 Schwarz J and Baum G L The history of histoplasmosis 1906 to 1956 *New England J Med* 56 2-3 258 1957

62 Schwarz J Silverman F N Adriano S M Straub M and Levine S The relation of splenic calcifications to histoplasmosis *New England J Med* 5 887 891 1955

63 Serviansky B and Schwarz J Calcified intrathoracic lesions caused by histoplasmosis and tuberculosis *Am J Roentgenol* 77 1034 1041 19

64 Steinberg B A Jambor W P and Suydom L O Amphotericins A and B two new antifungal antibiotics possessing high activity against deep seated and

superficial mycoses *Antibiotics Annual 1955 1956* New York Medical Encyclopedia Inc p 5 4

65 Straub M and Schwarz J Healed primary complex in histoplasmosis *Am J Clin Path* 5 27 41 19

66 Strong R P Study of some tropical ulcerations of skin with particular reference to their etiology *Philippine J S* 1 91 110 1906

67 Tenenberg D J and Howell A Jr Complement fixation test for histoplasmosis I Technic and preliminary results on animal sera *P l H alth Rep* 63 163 169 1948

68 Tompkins E H Personal communication

69 Van Pernis P A Benson M F and Holinger P H Specific cutaneous reactions with histoplasmosis preliminary report of another case *J A M A* 11 436 437 1941

0 Zarafonetis C J D and Lindberg R B Histoplasmosis of Darling observations on antigenic properties of causative agent preliminary report *Univ H sp Bull* Ann Arbor 7 4 48 1941

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF HISTOPLASMA CAPSULATUM

Leo Pine

The causal agent of the disease histoplasmosis was first observed and described by Dr S T Darling (14 15) who believed the organism to be a protozoon similar to the Leishman Donovan bodies of kala azar and gave the organism the generic name Histoplasma The species name capsulatum was used presumably to describe a capsular appearing halo which surrounded the small round or egg shaped bodies of the pathogen as they were observed in stained tissue sections Although da Rocha Lima (16) suspected the organism to be a fungus it was not until it was cultivated by DeMonbreun (17) and Hansmann and Schenken (30) that its true fungal nature was established DeMonbreun demonstrated the diphasic nature of the fungus by incubating fresh pieces of tissue at two different temperatures upon several media The yeast phase which is the phase observed in the tissues was obtained in an infusion broth incubated at 37 C whereas numerous *white cottony colonies* were obtained on Sabouraud s and dextrose agar slants incubated at room temperature Subsequently the cultural characteristics morphology and life cycle were described by DeMonbreun (17 18) Moore (47 48) Conant (10) Negroni (50 51) and Howell (32) Since no sexual state was established for the organism it was placed in the Fungi Imperfecti as a member of the family Moniliaceae (10) The life cycle of the fungus and its various structures and forms are diagramatically depicted in Figure 1

THE MYCELIAL PHASE

Cultural Characteristics of the Mycelial Phase For primary isolation from clinical materials the use of whole blood containing media will give the greatest number of positive cultures when the

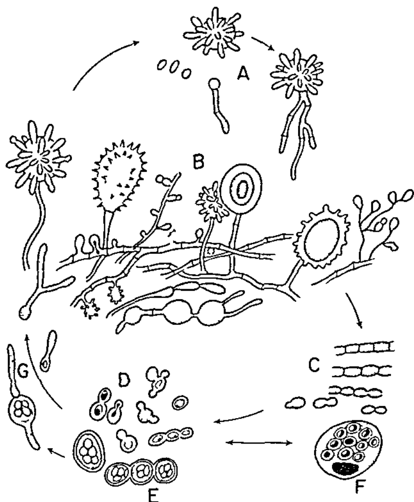


Fig 1 Diagrammatic representation of the life cycle of Histoplasma capsulatum A Tuberculate macroconidium and smooth microconidia in the process of germination B Various morphological forms of macroconidia microconidia chlamydospores and hyphal elements commonly observed in cultures of H capsulatum C Conversion of a hyphal fragment to yeast cells by the formation of moniliform chains D Actively growing yeast cells E Giant yeast cells found in old cultures F Appearance of yeast phase in tissue G Conversion of yeast cells to mycelial phase

cultures are incubated at 25 to 30°C (35 38 80) However the rate of growth of the mycelial phase on whole blood containing media is slower than on media without blood and the presence of blood inhibits the normal mycelial development of the fungus (Fig 2) When mycelial elements are transferred to media such as the Francis medium or brain heart infusion agar with blood and incubated at room temperature small white closely adhering colonies appear These gradually increase in size become raised in the center and remain tightly embedded in the agar at the periphery of the colony The colonies gradually darken become pink to tan in color assume a fuzzy verrucose appearance in the center and reach a diameter of approximately 6 to 8 mm in 14 21 days (Fig 2)

At 37°C the organism grows (if the strain does not convert to the yeast phase) as small cream colored glistening bacterial like colonies which are round convex hard and extremely adherent to the agar and with the complete absence of aerial mycelium As a colony increases in size it gradually assumes the color of the medium becoming dark brown with the character of the colony changing little In some cases the colony will become raised membranous cerebriform and will crumble easily when touched with an inoculating needle (Fig 2) Growth at 37°C is slower than at room temperature Also on blood media at 37 C sporulation and aerial growth of the mycelium are inhibited To promote sporulation it is necessary to make transfers to potato dextrose agar or Sabouraud s glucose agar and incubate the culture at 25 to 30°C (35)

When mycelial fragments are streaked over Sabouraud s glucose agar small white cottony colonies develop which increase in size at a rate which will invariably cover the slant within 14 days At the time maximum growth is reached the central portions and upper drier parts of the culture assume a yellow to buff color which slowly turns to tan or dark brown Gradually the entire culture will become brown in color and will take on a drier more fragile if not powdery appearance Usually but not always sporulation is associated with the tan coloration and aging of the culture Although growth in Sabouraud s glucose agar is greatly inhibited at 37°C some strains appear to adapt to the higher temperature after several passages (33 34) Coremia may be observed on infusion

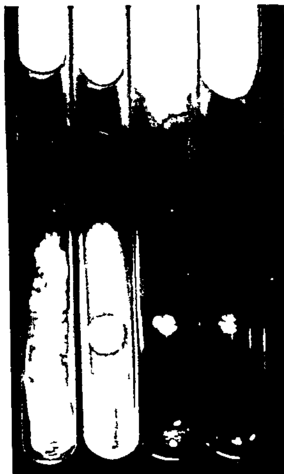


Fig 2 Growth of mycelial phase on Sabouraud's glucose agar and Francis blood agar slants. A One month old streak culture on Sabouraud's agar at 25 C B Seven day old colony on Sabouraud's agar at 25 C C Seven day old culture on Francis medium at 25 C D Fourteen day old culture on Francis medium at 37 C

tomato juice and brain veal agars at the higher temperatures (38 76) The colonial characteristics of H capsulatum on other media such as potato dextrose agar corn meal agar malt agar and nutrient dextrose agar will be essentially the same as that obtained on Sabouraud's glucose agar (18 50 17) Although continued use in

various laboratories has established the adequacy of Sabouraud's glucose agar for maintaining mycelial phase growth of H capsulatum certain inadequacies of the medium are recognized This medium is not the best medium for growth of small inocula Continued passage of stock cultures upon this medium invariably results in the loss of sporulation although some strains may be rejuvenated by transfer to a different medium In addition the medium is not selective and will support growth of other fungi to the disadvantage of H capsulatum (27)

Morphology of the Mycelial Phase In general the mycelium varies from 1 5μ in diameter is usually refractile branched and multicellular with each cell having one or more nuclei (50 48 32) When the mycelium becomes older its protoplasm is displaced toward the walls and vacuoles and oil globules are seen in the central portions of the cells At this time the walls thicken and a few cells may develop into peculiar swellings of assorted shapes and sizes which in some cases reach diameter as great as 15μ Racquet hyphae and occasionally coiled hyphae and nodular bodies may be formed while intercalary chlamydospores single and in chains and ranging from 5 to 8μ in diameter may be found in most cultures As the hyphae spread in radiating fashion from the center of a colony parallel hyphae lying at the periphery may be observed to undergo numerous anastomoses (Fig 3) These hyphal fusions do not result in the apparent formation of any specialized structures

Within 7 to 10 days after growth commences but dependent upon the medium conditions and strains sporulation begins These spores recognized as being aleurospores (32) are of two types macroconidia and microconidia The macroconidia are typically large tuberculate spherical to pyriform spores ranging from 10 to 25μ in diameter They are formed in the aerial portions of the mycelium generally at the end of short pedicels but may be sessile or formed at the end of long hyphae The tubercles may be 1 or 5 to 8μ in length (10 32 48 50) (Fig 1) It is this tuberculate spore which serves to identify the fungus as H capsulatum

As described by Howell (32) the large aerial spores begin their development as bulbous enlargements on the ends of lateral branches These branches may be simple with a single spore on the

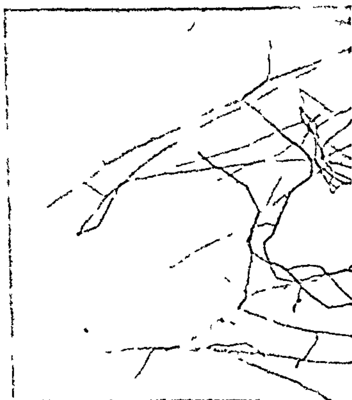


Fig 3 Anastomosis of hyphal elements (L A S stain—x00x)

end or divide into two to several short branches having a single spore on the end of each branch or one to several produced acropetalously and directly on a short branch As these spore initials increase in size they cut off from the rest of the hyphae by a cross wall near the base become spherical to pyriform in shape and the walls gradually increase in thickness

The character of the spore wall varies greatly and is dependent upon the strain examined the medium used or the part of the mycelium in which it is produced i e aerial or submerged In addition to tuberculate and smooth macroconidia there are forms having only several large bulbous or convoluted swellings or numerous spiny or warty projections (48 32 50) (Fig 4) The prepon

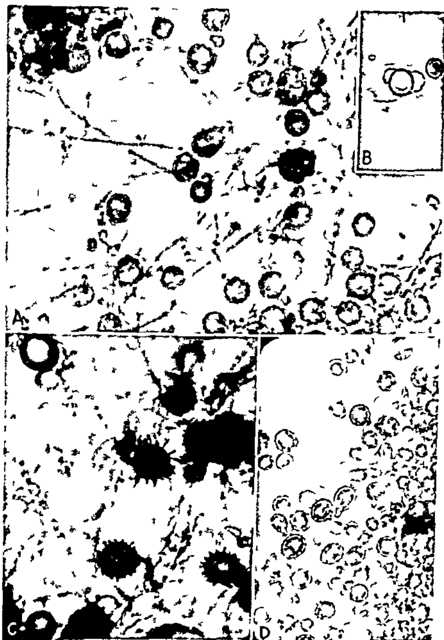


Fig 4 Macroconidia of four strains of Histoplasma capsulatum A Round and pyriform verrucose macroconidia (Lactophenol cotton blue—1000x)
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dence of smooth walled macroconidia has been noted deep in the mycelial mat next to or imbedded in the agar (32 18 50) Certain spores formed in these regions present a halo of substance surround ing the body of the macroconidium and have been called nymbospores (52)

The exact structure and chemical nature of the projections are not as yet clearly defined Negroni (50) believed the walls of the spores and the mycelium to be composed of callose although Nielson and Evans (52) did not find callose or cellulose in the sheath of the recent nymbospore Delve Scherre diagrams of the mycelium and yeast phases of the fungus have shown the walls to be composed of chitin There is no evidence of cellulose or any highly polymeric substance in the membranes (6) Moore (18) believed the pro jections to be devoid of protoplasm whereas Dowding (19) has described them as hollow extensions containing cell cytoplasm In view of the homogeneity of staining obtained with alkaline methyl ene blue cotton blue and periodic acid Schiff stains and the variety of forms of the projections exhibited by the same strain in a given culture and effect of different media on these forms it would appear that the tubercules of the macroconidium are formed by the excre tion of a metabolic product through pores in the cell wall of the spore The macroconidia themselves become filled with fat globules which may appear to be endospores but whose composition is readily recognized when adequate fat stains are used

Germination of the macroconidium may be induced under different conditions depending upon the strain (33) It may take place readily by simple inoculation into broth media (32) or may be induced by drying on cover slips for four to six days at room tem perature and then inverted onto agar media (17) Negroni (50) suc ceeded in inducing germination only when he soaked spores in solutions of trypsin or papain for 24 hours at 37 C germination was observed after 16 days Germination occurs with the formation of

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I Smooth walled and winged macroconidia (Lactophenol cotton blue—1000x) C Macroconidia with long and short clavate tubercle Note equal sized smooth macroconidia (P A S stain—1000x) D Spiny round and pyriform macroconidia Note large and virtually solid mass of spores (Lactophenol cotton blue—700x)

a single hypha which may branch immediately or which may continue a short distance prior to branching. Large swollen bodies may form at the end of the germ tube and these may again produce hyphae. As many as *three germ tubes have been seen to originate* from one macroconidium and these always arose from the same pole (32). Once formed the germ tubes grow rapidly, form cross walls, become vacuolate and spores develop in the culture within several days.

The microconidia are in general smooth walled spores, spherical, pyriform or even cigar shaped, being 2-6μ in diameter, sessile on short stalks or produced terminally or in dense clusters on extremely fine hyphae, the latter being less than a micron in diameter (Fig. 5). Some may be double celled or have buds; such conidia are capable of producing one or more secondary microconidia (19, 20). Upon closer examination one can often observe in a culture a small number of microconidial forms which duplicate the morphological characteristics of the various forms of macroconidia with the exception of size (Fig. 5).

According to Negroni (50) a microconidium has but one nucleus. During the formation of the microconidium a nucleus of the mycelial element from which the spore originates stretches and penetrates the spore initial. The nucleus then divides, one half remaining in the hyphal cell, the other half going to the microconidium. The nucleus is small and has an excentric nucleolus which may be demonstrated with an iron hematoxylin stain. A nuclear membrane is present, but no chromosomes were observed. The large tuberculated spore has not been studied.

It is emphasized that sporulation by Histoplasma capsulatum is dependent upon the strain of the organism, upon the medium on which it grows, and upon the environmental conditions of the culture. Some strains will sporulate so heavily, forming nothing but well defined large macroconidia, that at the time of maximum sporulation the mycelium appears to constitute but a small fraction of the total vegetation (Fig. 4). Other strains will form great numbers of microconidia and few if any macroconidia. In many strains there is a size and morphological differentiation between microconidium and macroconidium. In other strains, however, the gradation

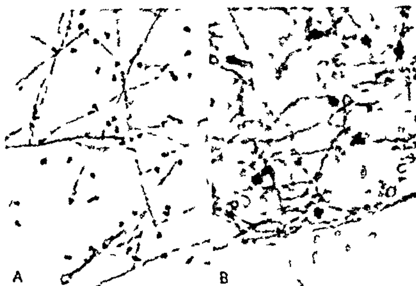


Fig 5 Microconidia of *Histoplasma capsulatum* A Gross appearance of microconidia (P.A.S. stain—[illegible]00x) B Various forms of microconidia Note folded clavate septate spores and verrucose forms (P.A.S. stain—1000x)

of size of the spores progresses so smoothly from microconidium to macroconidium that no real distinction can be made between the two forms. It would appear that in these latter strains the microconidium is but an immature macroconidium (32, 40). A strain which may form tuberculed spores on one medium may form smooth spores on a second medium.

In general, however, it is the small spore which predominates. Conant and Furcolow (12) observed that the vast majority of the spores of 11 strains were less than 5μ in diameter. Similar results were obtained with five of these same strains by Helmbrecht and Larsh (31). These results are consistent with a theory of airborne infections in histoplasmosis since particles greater than 5μ rarely reach the alveoli (8).

From a diagnostic standpoint it is particularly fortunate that virtually all of the strains form the characteristic macroconidium on primary isolation from clinical material. However, few of the specific factors responsible for the loss of sporulation on continued

cultivation in the laboratory are known. The addition of whole blood and temperatures greater than 32°C inhibit sporulation (33). The effect of a relatively high temperature may be due to its inhibition of growth. Similarly, the fact that some strains sporulate better at pHs ranging from 6.5 to 7.5 may be correlated with greater growth of the fungus at these pHs (34). However, better sporulation is not always a result of better growth. Negroni (50) has shown that sporulation may be greatly affected by the carbohydrate or nitrogen of the medium. For example, in a glucose medium source asparagine supported best growth (+++) at room temperatures but only macroconidia formation. KNO_3 gave less growth (+) and many smooth and rough macroconidia with microconidia. $(NH_4)_2SO_4$ gave (+) growth with only smooth macroconidia and microconidia, and when no nitrogen source was added he found abundant microconidia and a few *smooth macroconidia with (+) growth*.

Subsequent to the demonstration by Emmons (26) of the natural occurrence of macroconidia in the soil, investigations by other workers have shown the relative high occurrence of Histoplasma in soils associated with chickens (82). The chickens themselves are free of the disease. Unfortunately, analyses of certain of the physical and chemical characters of such soils did not reveal any marked difference between soils containing Histoplasma and soil from which the fungus could not be isolated (83). The factors stimulating growth and sporulation in such soils are as yet unknown.

The spore is resistant to drying and will remain viable in dry soil for as long as four years (58). They are resistant to a temperature of 15°C for 30 minutes (17, 50). Other strains suspended in milk were resistant to temperatures of 62 to 63°C for 10 minutes (4). Kao and Schwarz (36) tested 7 strains for their resistance to heat. All strains were killed at a temperature of 50°C for 1 hour or at a temperature of 60°C for 5 minutes. There was no correlation of pathogenicity with relative heat resistance. The spores will survive as long as 600 days in water at 4°C but rapidly undergo a decrease in viability as the temperature is increased to 37°C at which the maximum survival time was 62 days in tap water (11, 60). Spores exposed to 1/1000 or 2/1000 formaldehyde for 24 hours did not survive, whereas occasional spores survived these dilutions of phenol (50).

However, viability of the spore is a function of the medium used for determination of colony counts. Grayston *et al.* (28) obtained 15% viable spores when the spores were plated on corn meal agar, 29% on Sabouraud's agar, and 12% on blood agar. Larsh *et al.* (40) used four strains of Histoplasma which had 11 to 89% viability of their spores as measured by colony counts. In mice, however, all strains produced 100% infections even though small numbers of spores were used. Ajello and Runyon (1) obtained infection with 94 and 100% of the single tuberculate spores which they introduced into the abdomen of mice.

In general the mycelial phase of the organism is not found in the tissues. However, on one occasion Haley (29) observed hyphae and tuberculated macroconidia in sections of a necrotic lesion of the liver of a mouse which had been inoculated with the yeast phase. The lesion had erupted through the capsule of the liver. Binford (5) points out that mycelial elements occur in the sites exposed to body fluids and those sites most distant from viable tissue (Fig. 10). Usually the mycelial and swollen forms are found in tissues occurring in old necrotic lesions. In two cases with valvular lesions exuberant mycelial growth occurred on or near the surface of the thrombus (5).

Physiology of the Mycelial Phase. The optimum pH found for growth of the mycelial phase on a synthetic medium was approximately pH 6.5 (33). At pH 5.5 to 6.5 considerable aerial mycelium and macroconidia were formed on this medium whereas at pH 7.7 to 8.6 there was little or no aerial mycelium and sporulation was negligible. At 37° C on Sabouraud's broth the optimal growth of 5 strains occurred at pH 6.8 to 6.9 (13). The optimum temperature for growth ranges from 25 to 30° C, and temperatures greater than 32° C are usually inhibitory (33). Based on optical density measurements, a generation time of approximately 12 hours has been obtained at 25° C on shake culture (56). A high humidity is recognized as usually being beneficial on agar media. A 100% humidity was required to obtain mycelial growth on elm bark at 20 to 25°C while no growth was obtained at these temperatures at 98% humidity (45). The organism is a strict aerobe and growth will be greatly inhibited by merely stoppering the culture tube with rubber

stoppers or by growing it beneath the agar surface (17)

The fungus will grow very well on a variety of synthetic media or media containing gross plant or animal extracts To date few vitamins have been observed necessary for growth Area Leao and Cury (2) found one strain of three which required thiamin for growth Scheff (68) obtained no growth on the second transfer in the absence of thiamin and reduced growth in the absence of riboflavin biotin inositol niacin or pantothenate Salvin (66) found a single strain of six tested which required biotin for maximum growth when alanine was the only nitrogen source However Pine (56) observed no diminished growth when the mycelial forms of 12 strains were grown in the single or combined absence of vitamins

Salvin (66) found no single amino acid was required for growth Furthermore the six strains tested grew well on asparagin acetamid and glucosamine as sources of nitrogen In addition to some of these nitrogen sources Negroni (51) previously had reported good growth on histidine cystine urea and peptone with histidine and peptone supporting the best growth The earlier results of Negroni regarding the effect of nitrogen source on sporulation have been mentioned above

Of the carbohydrate sources glucose is perhaps the substrate of choice Negroni (51) found excellent growth on glucose galactose mannose and mannitol with one strain and less growth on *maltose lactose raffinose trehalose dextrin sorbitol inulin* sodium oxalate sodium tartalate and sodium citrate Sodium acetate did not support growth Scheff (68) obtained the following dry weights of mycelium with various sugars glucose—164 mg maltose —387 mg galactose—55 mg lactose—183 mg sucrose—205 mg and starch—70 mg Furthermore only when glucose was used as a substrate did he observe acid formation Using 3% solutions of the various sugars in Durham tubes Negroni (50) observed no acid or gas formation with glucose galactose fructose mannose lactose maltose sucrose raffinose inulin or starch However Howell (34) has observed acid formation from glucose sufficient to lower the pH from 6 5 to 3 8 The final pH formed was found to be a function of the amino acid to sugar ratio the smaller the ratio the less the drop in pH The results indicate utilization of the amino acids for

growth and energy with the subsequent release of ammonia. Scheff (68) found that the fungus reduced the amide nitrogen of asparagine slowly at first and more rapidly later. The amino nitrogen fraction of the molecule was also released. Growth was stimulated by succinic and fumaric acids, with fumarate being the better of the two. Stimulation in the presence of glucose was merely additive. Pine and Peacock (57) observed stimulation of mycelial growth with alpha-ketoglutaric acid and inhibition of mycelial growth by citrate. The inhibition by citrate was apparently due to chelation of essential metals since the addition of calcium or magnesium salts reversed this defect.

The mycelial phase liquifies gelatin very slowly if at all, does not attack cellulose, does not hydrolyze albumin, has no action on milk, does not form hydrogen sulfide, acetoin or indole, but does reduce nitrates to nitrites, and hydrolyzes fats (50). Starch is not hydrolyzed.

It is seen that little is known regarding the metabolism and metabolic pathways of the mycelial stage. However, from the information on hand it would appear that this phase of the organism has little proteolytic activity and that its primary source of energy for growth comes from the oxidation of sugars or dicarboxylic acids, although free amino acids may also be used. Although it is agreed that the mycelial phase is readily grown at 25 C on a variety of media with relatively few nutritional requirements, the need for additional growth factors at 37 C is strongly indicated (13, 70, 71). However, generalizations should be limited for each strain has its own peculiar characteristics regarding its sporulation, nutritional requirements, responses to temperature and other environmental conditions.

Conversion of the Mycelial Phase to the Yeast Phase. When DeMonbreun first isolated H. capsulatum, he observed that both mycelial and yeast phase elements grew in the infusion broth inoculated with the clinical material (17). If the yeast phase was then transferred from the broth to blood agar tubes and maintained at 37 C, it would grow and could be maintained in the absence of the mycelial phase. However, to convert pure mycelial stock cultures to the yeast phase, it was necessary for DeMonbreun to inoculate

animals and again isolate the yeast phase on blood agar plates at 37°C Negroni (50) in attempting to stimulate germination of the macroconidium soaked the spores in sodium hydroxide and sodium borate solutions He observed that when his suspension was treated with 0 5% sodium borate for 24 hours at 37°C then washed to remove the excess borate and transferred to blood agar yeast cells were formed after 12 days at 37°C Conant (10) reported conversion of the mycelial phase to the yeast phase in vitro by streaking the mycelium on blood agar slants incubated at 37°C with paraffin seals Conversion occurred in 7 to 10 days Campbell (9) described the conversion to yeast phase by transferring every two to three days on paraffin sealed tubes of the Francis glucose cystine blood agar medium The cystine was apparently the additional factor responsible for the increased ease of conversion Thereafter, other investigators reported various procedures or media for obtaining conversion to the yeast phase (39 41 58 75) Titsworth and Grunberg (75) were the first to recognize the value of an inspissated egg starch medium for conversion of mycelial phase to yeast phase Using their medium they were able to convert 23 strains to yeast phase in one or two transfers Because of its ease of preparation the Kurung Yegian medium (39) is perhaps the most useful of the conversion media This medium will generally give pure yeast phase after several transfers at 37°C and is an excellent maintenance medium A small amount of nutrient broth may be added to moisten the slant and to stimulate yeast phase growth Larsh *et al* (41) described the successful conversion and maintenance of Histoplasma in HeLa cell tissue cultures

The factors involved in inducing the conversion of the mycelium to the yeast phase in living tissues are not known Although conversion of the yeast phase to mycelial phase will occur very readily by merely transferring the yeast to a non blood medium at 25°C conversion in vitro from mycelial phase to yeast phase is still induced with great difficulty Some strains will resist all efforts to obtain such a conversion Inoculation into a susceptible animal however is virtually always a successful method of obtaining the yeast phase (10)

Recently some of the factors involved in converting the my

celial phase to the yeast phase were investigated (58). Satisfaction of yeast phase growth requirements is not sufficient in itself to induce conversion. For example, a blood medium which supports a good rate of growth of yeast phase may fail completely to induce conversion. Continued transfer of the mycelial phase of such a strain at 37 C merely promotes better mycelial growth at this temperature. On the other hand, the inspissated egg starch medium may stimulate conversion of mycelial phase to the yeast phase even though the rate of growth of the yeast phase is slower and more atypical on this medium than on blood agar media (58, 22). A temperature of 37 C is generally assumed to stimulate conversion to the yeast phase by inhibiting the growth of the mycelium. However, Myers and Sherwood (19) have reported conversion to the yeast phase in a tree frog maintained at 25 C. Pine and Peacock (58) obtained conversion with some strains at 30 C in vitro. In addition, chelating agents such as citric acid, glycylglycine, or versene stimulated conversion of the mycelial phase to the yeast phase. If calcium ions were added to the medium the citrate effect could be reversed and good mycelial growth would occur at 37 C, while the addition of magnesium or zinc ions stimulated the conversion to and growth of the yeast phase of several strains. Even though the effects of a temperature of 37 C and chelation of metal ions by serum albumin, citrate, and other body substances might be visualized as occurring in the animal body, it is probable that other factors more important than these are responsible for limiting mycelial growth in the animal and in stimulating conversion to the yeast phase.

The morphological sequence of events which lead to the formation of yeast phase in tissue have not been clearly elucidated (46). In general, subsequent to the introduction of the mycelial elements into the animal, one observes only pure yeast phase in the tissues. Sometimes the ungerminated macroconidia or mycelial elements may be found at the site of the inoculation (16), but mycelial growth itself occurs rarely and usually under the specific conditions described by Binford (5). However, in vitro the cells of the hyphae may undergo the series of morphological changes to form yeast-like cells as depicted in Figure 1. The individual cells swell in the center but remain constricted at the cross walls until moniliform chains

are formed These fragment easily forming individual oblong yeast like cells which then bud usually at either pole but occasionally from non polar areas Dowding (19 20) has observed the formation of yeast cells from the various mycelial elements At 37°C the microconidium was seen to enlarge and form a thin walled yeast cell with granular contents Although the formation of yeast cells from the tubercles of the macroconidia was also reported this has not been confirmed It appears therefore that the yeast cell may also be formed by budding of the hyphal cells or microconidia or by germination of the spores with the formation of short germ tubes The latter form the yeast cell by direct budding or from moniliform hyphae

THE YEAST PHASE

Cultural Characteristics of the Yeast Phase Of the various media which have been described for the yeast phase (9 65 67 75 81 39 42 7 58) those media containing whole blood are recognized as being of the greatest general laboratory value For this reason the factors responsible for good yeast growth on blood agar media should be noted

DeMonbreun (17) observed that drying of the medium caused many yeast colonies to revert to the mycelial phase and that a high degree of moisture maintained by sealing the tubes with paraffin could prevent this However with a fresh 1 5% agar medium sufficient moisture is present and it is not necessary to seal the tubes if the incubation periods are to be of approximately one week duration A temperature of 37 C is essential since lower temperatures stimulate conversion to the mycelial phase

The value of cystine in blood media was indicated by the observation of Campbell (9) and supported by the work of Salvin (66) although good growth of the yeast phase on a blood agar medium may be obtained in the absence of added cystine (12)

DeMonbreun also emphasized the need for small amounts of serum to initiate yeast phase growth in infusion broth at 37°C Subsequently useful laboratory media containing albumin or serum have been described by Zarafonetis (81) and Bonorden (7)

By fractionating the components of whole blood McVickar (44) observed that serum albumin removed toxic factors in agar media which inhibited the growth of the yeast phase Pine (54 55) presented evidence that albumin maintained non toxic concentrations of long chain fatty acids which were also required for growth of small numbers of yeast cells

The whole viable red cell was found to be required for the maximum beneficial effect of a blood medium Plasma by itself gave only 10% of the total stimulation of whole blood washed red cells 52% and the reconstituted blood gave 93% of the activity of the whole blood Whole blood heated for 15 minutes at 100 C did not support growth of the yeast cell and the addition of lysed red cells had only 24% of the activity of whole blood in the presence of serum (55) However as discussed earlier even in the presence of whole blood colony counts obtained with a small number of yeast cells incubated at 37 C will generally be substantially less than those obtained at 25 C (35 38 55 80)

In general the use of the Francis blood glucose cystine medium prepared as described by Rowley *et al* (61) will support satisfactory growth of yeast cultures at 37 C However several lots of the beef heart infusion base should be tested if the medium prepared does not give the desired results Some lots of commercial or freshly prepared base are strongly inhibitory to the growth of the yeast phase and the growth obtained will be cerebriform wrinkled or granular with a substantial amount of mycelial phase present Similarly blood which is black with age partially lysed or which has preservatives should not be used Blood which is defibrinated or citrated is satisfactory Of many media tested Littman (32) found that a liver spleen extract medium with whole blood supported the best growth and development of colonies from small numbers of yeast cells

When transferred to freshly prepared blood glucose cystine agar slants and incubated at 37 C the yeast phase growth will appear on the second or third day as white or cream colored round convex colonies These gradually increase in size and coalesce forming a moist raised butyrous growth whose surface may be rough to granular or very mucoid (Fig 6) Maximum growth is reached

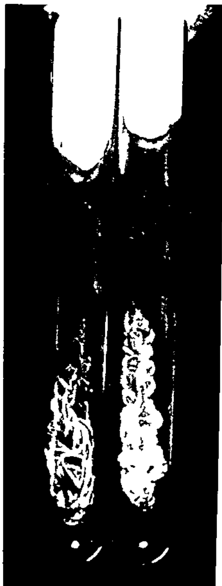


Fig 6 Yeast phase of Histoplasma capsulatum Two types of yeast phase growth obtained by different strains of H capsulatum incubated on Francis medium for 5 days at 37 C

on the seventh or eighth day. Subsequently the culture becomes darker, assuming the red brown or chocolate color of the medium. On a blood medium which supports good growth of the yeast phase frequent transfers need not be made to maintain stock cultures. Instead, the cultures may be incubated at 37 C for a sufficient time to allow heavy but not maximal growth and then transferred to a 5 C refrigerator where they may be stored for as long as a month without loss of viability or change in phase.

Similarly, one may use the Kurung and Yegian (39) (or Tits worth and Grunberg (75)) inspissated egg and starch medium for maintenance of stock yeast cultures. 0.5 ml of nutrient broth is added to maintain moisture and yeast phase growth occurs within 5 to 9 days, usually just above the fluid line. When stored at 4 C on this medium the yeast was found to be viable after 9 months while those stored at room temperatures were viable at 3 months, at which time they were discarded. In addition, this medium may be used to convert the mycelial phase to the yeast phase. In some cases several transfers may be required to obtain yeast phase growth devoid of mycelial elements.

Morphology of the Yeast Phase. The yeast phase cells of young cultures are oval bodies which are approximately 1.5 to 2.0μ by 3.0 to 3.5μ (Fig. 7). In actively growing cultures one pole of the cell is pointed; buds may be formed at either pole and in some cases bud formation is apolar. As many as three buds may be formed simultaneously from a single mother cell. The cells usually appear round or oval but elongated forms, swollen forms and dumbbell shaped cells are not unusual. Cells have a fine cell wall within which small refractile oil droplets may be observed with one or more granules in active Brownian movement. In preparations stained supravitally with dilute methylene blue the protoplasmic granules stain very intensely revealing one or more vacuoles. In stained preparations the nuclei of the cells usually appear as peripheral crescent shaped masses (17). The single nucleus of a budding cell approximates itself near the bud, constricts and divides with one half going to the daughter cell (50). A halo about the cells may appear to be capsular substance but dilute india ink preparations or Hiss capsule stained preparations do not reveal a true capsule (10). Kligman and Bald



Fig 7 Yeast phase of Histoplasma capsulatum from blood agar slant (Methylene blue vital stain—1000x)

ridge (37) using the electron microscope were unable to demonstrate the presence of a capsule *in vitro* or *in tissues* Ribi and Salvin (59) also were unable to find any electronmicroscopic evidence for the presence of a capsule on cultured cells although their methods allowed easy detection of the capsule of Cryptococcus neoformans In cultures which are a week or more old large swollen forms may be observed which are from 2 to 3 times the size of actively growing forms and which possess a thick wall and one or more large internal glubules of fat These forms are resistant to drying and may remain viable for as long as two months at room temperature They will



Fig 8 Yeast phase of Histoplasma capsulatum from impression smear of spleen (A Wright stain—1000x B Wright stain—1800x)



Fig 7 Yeast phase of Histoplasma capsulatum from blood agar slant (Methylene blue vital stain—1000x)

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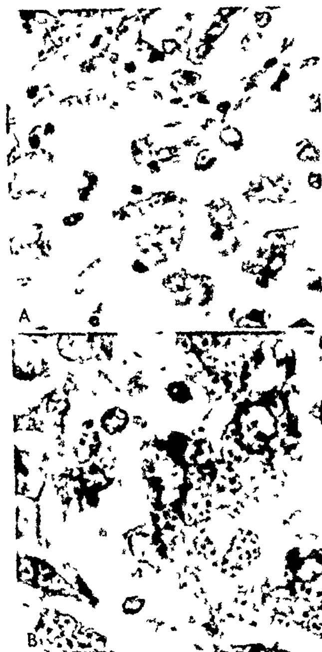


Fig 3 Yeast phase of Histoplasma capsulatum in lymph node (A P A S stain—1000x B 1800x)

withstand a temperature of 45°C for thirty minutes but are killed when subjected to 55°C for this period of time (17)

Yeast cells observed in tissues are somewhat smaller in size than those observed in culture Depending on the stain the tissue forms are from 1 to 4μ in diameter and are surrounded by a definite halo suggestive of a capsule In light of the results of Kligman and Baldridge (37) and Ribi and Salvin (59) this capsule is considered to be an artifact resulting from the shrinkage of the protoplasm within the yeast cell wall (5) When stained with Giemsa or Wright s stain a pale light blue ring surrounds the darker blue of the cell protoplasm (Fig 8) The violet chromatin material appears as an oval half moon or crescent shaped mass within the center With the hematoxylin eosin stain usually only the granular appearance of the central protoplasmic mass is observed within the halo and internal structure is not observed Stained with the periodic acid Schiff stain the cells appear as round or oval cells with extremely fine and delicate cell walls (Fig 9) The cell wall takes a light pink or purple stain Within the cell the central protoplasm appears as a solid or finely outlined mass which duplicates the external shape of the organisms Within the protoplasm heavier staining material may appear as an eccentric hemispherical oval or crescent shaped violet or purple body Although budding is rarely observed with the above stains it is more easily observed when the Gomori modification of the silver nitrate methenamine stain is used (3) With this stain the *purple black to black outline of the fungus wall stands out in strong relief* against the background tissue and duplicates the morphology of the cultured yeast phase

Larger forms of the fungus described by DeMonbreun in old cultures have also been found extracellularly in old necrotic tissue (5) (Fig 10) They stain poorly if at all with hematoxylin and eosin but will stain well with the periodic acid Schiff and the Gridley stains Although they reach the size of 15 to 20μ in diameter little internal structure is seen with any of the stains Schwarz (73) observed such large forms in tissue which had been incubated at 37°C on blood agar plates

Large forms of the yeast phase have been noted in culture since DeMonbreun isolated the organism and in general laboratory dis

many yeast forms larger than those observed with H capsulatum Subsequently the number of duboisii forms increased until at two months after inoculation this form predominate Subsequent conversion of the mycelium to yeast phase on the Kurung and Yegian medium showed that in general the yeast cells of H duboisii were larger than those of H capsulatum with many forms 13 to 15μ in diameter (25)

Of the various criteria (78 79) used to distinguish H duboisii as an entity separate from H capsulatum two appear to be of most value First the duboisii form is reported to be primarily intracellular and not restricted to necrotic areas as reported by Binford (5) for some large forms of H capsulatum Secondly the large duboisii form is the only recognized parasitic form in man and is also the only one to be found in animals some weeks after inoculation In addition there appears to be a definite morphological difference between the large irregular forms of H capsulatum found in tissue and those of H duboisii Drouhet and Schwarz (21) were unable to separate H duboisii and H capsulatum following studies of morphology and virulence in mice and hamsters or by gross appearance rate of growth or pigmentation on various media (22) Later the results of histological studies done on mice and hamsters experimentally infected with a strain of H duboisii were in agreement with the results of Vanbreuseghem (77) and the organism could be differentiated from American strains of Histoplasma on the basis of its virulence and morphology in tissues (Fig 11) The fact that 15 known or potentially recognizable cases of African histoplasmosis have occurred (77) emphasizes the importance of H duboisii as a possible new species (43 71)

Physiology of the Yeast Phase Growth of the yeast phase is strictly aerobic (17 51) and in a liquid medium the rate and amount of growth in stagnate tubes is much less than that obtained in shake tubes using the same medium In liquid shake tubes the generation time may range from 9 to 11 hours under optimal conditions (58) while on blood agar media the generation time varies from 6 to 8 hours (62 55) Rowley and Huber (63) inoculated mice with nonlethal doses of the yeast phase harvested the mice at various time intervals and made total colony counts from the various tissues

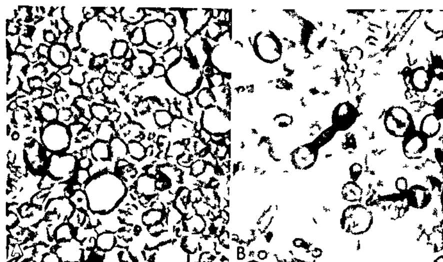


Fig 10 Yeast phase of Histoplasma capsulatum A Giant forms at edge of necrotic area of thrombus aortic valve B Giant forms bumbell forms and mycelial elements deeper within necrotic area (From Binford C H Histoplasmosis Tissue reactions and morphologic variation of the fungus *Am J Clin Path* *25* 25 36 1955 Courtesy of Armed Forces Inst Pathology Washington D C)

cussions are sometimes referred to as degenerate or giant forms Dowding (20) Weed (80) Schwarz (73) have all emphasized the close resemblance of these heavy walled forms to the yeast cell of Blasto myces However recently these forms have been given a new significance since they are believed to be a true tissue phase in the life cycle of a strain of Histoplasma isolated from African Histoplas mosis (23 24) This isolate has been named Histoplasma duboisii

Mycologically the mycelial form of this fungus is identical to and can be duplicated by any strain of H capsulatum However in the lymph nodes of a patient from which cultures were isolated the yeast cells were ovoid 10 by 13 5μ in size They had a double wall 1 to 2μ thick and contained a very large amount of fat as a single perfectly round globule or as numerous small globules which filled the cell almost completely Numerous budding cells were also observed When inoculated intratesticularly into Guinea pigs numerous capsulatum forms of the organism were observed after 8 days At three weeks testis punctures revealed the presence of

many yeast forms larger than those observed with H capsulatum Subsequently the number of duboisii forms increased until at two months after inoculation this form predominate Subsequent conversion of the mycelium to yeast phase on the Kurung and Yegian medium showed that in general the yeast cells of H duboisii were larger than those of H capsulatum with many forms 13 to 15μ in diameter (25)

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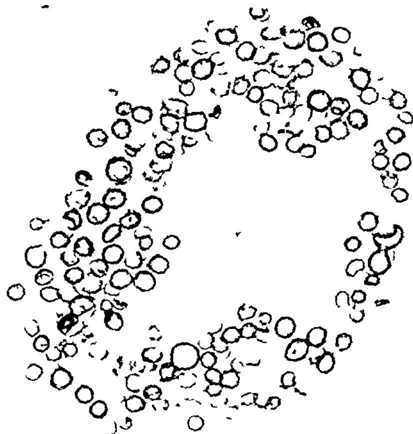


Fig 11 Tissue phase of African strain of Histoplasma duboisii in hamster liver Large yeast cells within giant cell Majority of cells are larger than 10μ Note budding cells at the left and at the top of the field (Courtesy Schwarz and Drouhet Morphologic features of an African strain of Histoplasma in hamsters and mice *AMA Arch Path* 64 109 113 1957)

Logarithmic growth of the yeast phase was observed in the animals from the first to fourteenth day with an estimated generation time of 15 to 19 hours Since single colonies undoubtedly grew from macrophages containing many yeast cells the generation time of the yeast phase of Histoplasma in tissues is probably less than 15 hours

In general the growth of the yeast phase on blood agar or serum media occurs at temperatures between 34 and 37°C At lower temperatures conversion of the yeast phase to the mycelial

phase generally occurs. However, yeast phase growth may be maintained at temperatures of 25 C (56, 70). There has been no report as to the optimum temperature determined within small increments for yeast phase growth on a given medium. Nevertheless, the results of Scherr (71) suggest that this is less than 30 C since greater amounts of yeast phase growth occurred at 25 C. Cysteine was required for yeast phase growth at 25 C (71). Other workers have also found that a temperature of 37 C is inhibitory to the yeast phase growth (56, 58). At 37°C the yeast phase is more sensitive to vitamin deletions than it is at 25 C and the rates of growth of several strains are greater at 25 C if not equal to those at 37 C. Salvin (65) found that growth of the yeast phase at 43 C was less than that obtained at 37 C. Negroni (50) found that the yeast phase did not survive 15 minutes incubation at a temperature of 55 C. Therefore, it would appear that the value of a temperature of 37 C lies not in its stimulation of the yeast phase growth but in its inhibition of yeast phase conversion to mycelial phase and the subsequent inhibition of mycelial phase growth.

The optimum pH for yeast phase growth is considered to be between 6.5 and 7.5. Cross (13) tested the effect of pH on yeast phase growth in three different media. In Sabouraud's broth all growth was mycelial regardless of pH. On beef extract broth the best growth was from pH 7.0 to 7.3. All strains showed a tendency to convert at pH 6.7 to 7.0 and 7.5 to 7.9. Best yeast phase growth was supported by brain heart infusion and this was at pH 7.2 to 7.6. Salvin (65) found the maximum growth of the yeast phase occurred between pH 6.3 and 8.1. On a basal agar medium with added whole blood the rate of growth of one strain of H. capsulatum was the same at pH 5.5, 6.5 and 7.5 (54). However, with serum albumin substituted for blood the rate of growth increased with increase in pH. Yet the numbers of colonies obtained from small numbers of yeast cells were much lower at pH 6.5 than at 4.5. The rate of growth of the yeast phase on a synthetic medium increased from pH 4.5 to 6.5 but no growth was obtained at pH 7.0 and 8.0. These results were interpreted as suggesting a requirement for cysteine (54).

As a carbon source for growth Negroni (50) found glucose, mannose, mannitol, or inulin supported the best growth while

growth on starch was somewhat poorer Fructose galactose lactose maltose and sucrose did not support good growth and no growth occurred on xylose Of several sugars and polysaccharides tested Pine (54) found that glucose and glycerol were the best substrates for growth of the yeast phase *Small additions of organic acids in* the presence of glucose did not stimulate growth although the addition of pyruvate and fumarate inhibited growth Subsequently it was found that as a single substrate alpha ketoglutaric acid supported a high rate of growth of 10 strains By itself citrate supported good growth of only five strains whereas in combination with glucose a high rate of growth for all strains was noted (58)

Negroni (50) failed to obtain growth on a synthetic medium with 0 1% amounts of potassium nitrate ammonium sulfate asparagine or peptone as the sole nitrogen source Salvin (66) observed extensive growth of the yeast phase only in the presence of amino acids or proteose peptone The development of the yeast phase did not occur to any great extent on ammonium nitrate purines uracil ammonium sulfate urea or acetamide On a synthetic amino acid mixture Salvin (66) was unable to demonstrate the requirement for any single amino acid However when single amino acids or tripeptides were used as the nitrogen source only cystine cysteine and glutathione supported growth In the presence of non sulfur amino acids cystine cysteine methionine methyl cystine and dl homocystine supported pure yeast phase growth whereas sodium thioglycolate glutathione diglycylcystine and NaHS supported a mixture of mycelium and yeast Subsequently Pine (54) observed that the maximum rate of growth for *five strains occurred on a mixture of glutamic acids* aspartic acid and cysteine In the presence of glutamic and aspartic acids methionine would not substitute for cysteine and in the presence of casein hydrolyzate glutathione likewise would not substitute for cysteine In a defined medium containing glutamic and aspartic acids equal rates and amounts of growth were obtained with 0 1 and *0 05% cysteine while no growth was obtained with 0 025%* cysteine Analyses for cysteine-cystine showed that these completely disappeared from the medium during growth On the basis of these results the results obtained with changes in pH known reac

tions of cysteine with pyruvate (72) and with fumarate (57) it was suggested that both the amino acid cysteine and the –SH radical were required for growth of the yeast phase. Scherr (71) has found that cysteine is required to maintain yeast phase growth at 25°C. The exact role of the –SH radical in promoting the growth of the yeast phase is not known, although this requirement also is known to exist in other dimorphic fungi (69, 53).

The vitamins necessary to induce growth of a relatively large inoculation of the yeast phase will vary with the type of assay used, assay medium, and strains tested. Salvin (66) demonstrated a general requirement by eight strains of yeast phase cultures for approximately 1.0 millimicrogram of biotin per ml when cysteine was the sole source of nitrogen. Desthiobiotin or o-heterobiotin would not substitute for the biotin requirement. Therefore the sulfur in biotin would appear to be essential under these conditions. Subsequently, it was found that eight of 11 strains required thiamin (approximately 0.02 micrograms/ml) for maximum rate of growth in a casein hydrolyzate medium, one strain required biotin and a second was stimulated by thioctic acid (56). These requirements, however, were not absolute since most of the strains were capable of continued growth for as many as four transfers in the absence of added vitamins; some strains required 3 or 4 transfers before any deficiency was noted. Carbon dioxide has been observed to be stimulatory under certain conditions (65, 54) but not under others (55).

It is generally recognized that small numbers of yeast cells are sensitive to external factors and under these circumstances the organism is relatively difficult to grow. Plating experiments on agar media show that when an inoculum is reduced to 500 cells or less, few colonies appear at 37°C even though these cells are capable of growing as mycelial colonies at 25° C (55). Rowley and Huber (62) found that the viability of the yeast cell decreased rapidly when suspended in physiological saline at 25° C, whereas at 5° C the viability was maintained for 4 hours. Sixty per cent of the cells suspended in saline having 0.1% cysteine still retained their ability to grow into mycelial colonies at 25° C after maintenance for 24 hours at 4°C. Only 35% remained viable in the absence of cysteine. When small numbers of yeast cells were inoculated into stagnate liquid

tubes oleic acid was required to initiate growth of some strains (54) However when the tubes are shaken no growth will occur unless the inoculum is increased to approximately 500 000 cells per ml (54 71) The factors necessary to initiate growth of smaller inocula in shaking tubes are unknown The need for —SH may reflect the requirements for a lowered oxidation reduction potential (71) or serves in some unknown manner to maintain the viability of the cell until growth commences Of the factors which stimulate growth of the yeast phase Salvin (65) was the first to observe the beneficial effects of small amounts of agar and silica gel Subsequently the value of agar albumin and starch have been noted (44 81 54 64 55 71) Although these may function to bind toxic fatty acids and make these acids available for growth it should be recognized that these substances and cysteine are strong metal binding substances Therefore their stimulation may be a consequence of chelation and not a result of detoxification or satisfaction of nutritional needs

The yeast is non fermentative in its growth on carbohydrates and forms neither acid nor gas from a variety of sugars The organism reduces nitrates to nitrites but does not form indole or acetyl methyl carbinol does not hydrolyze starch cellulose or affect milk (50)

In a general way the yeast phase may be described as a more sensitive phase than the mycelial phase and one whose growth factor requirements are more stringent than those of the mycelial phase It is apparently more adapted for growth at 37 C Although all the growth requirements are not known or understood there is a strong suggestion that the yeast phase is deficient in its ability to synthesize sulfur containing growth factors since cysteine-cystine biotin thiamin are the only compounds that have been demonstrated as general requirements It too is a strong aerobe which preferentially oxidizes sugars and carboxylic acids for growth It forms no extracellular toxins So far there is no single absolute major physiological criterion known for distinguishing this phase from the mycelial phase with the exception that the yeast phase is the phase most capable of significant growth in the mammalian host

Conversion of the Yeast Phase to the Mycelial Phase Conversion of the yeast phase to mycelial phase is generally accomplished

by simply dropping the incubation temperature of the yeast phase from 37 to 25 C Using slide cultures and Van Tieghem cell cultures at room temperature Conant (10) described the morphological changes which occurred in conversion to the mycelial phase Within 24 hours the yeast cells became swollen reaching a size of 3 5 x 5 5μ and within 48 hours had produced short germ tubes The germ tubes originated from the pointed end from both ends and occasionally from the sides Occasionally three germ tubes from a single cell were observed The germ tube soon became septate branched and showed a highly vacuolate protoplasm and numerous oil droplets The subsequent development and characteristics of the mycelial phase have been described earlier in this chapter

Rowley and Pine (61) have reported on some of the nutritional factors concerned in the conversion of the yeast phase to the mycelial phase The numbers of colonies originating from 100 cell aggregates of one strain was much less in the absence of calcium pantothenate However with three other strains no differences in colony counts were observed in the presence or absence of pantothenate Similar studies showed that glutamic acid isoleucine lysine proline or serine were implicated in the conversion of one or more of the strains Phosphate was found to be inhibitory in the medium and this inhibition could be reversed by the addition of starch These results suggest that the converting organism has greater nutritional requirements than the subsequent mycelial phase and requirements different from that of the yeast phase However it should be considered that this is perhaps a more delicate assay for nutritional requirements than can be accomplished for the yeast phase growing as yeast colonies Such requirements may have been hidden by the assay methods used for the yeast or mycelial phase

REFERENCES

1 Ajello L and Runyon L C Infection of mice with single spores of *Histoplasma capsulatum* Pub Health Monogr No 39 19 6 pp 93 98

Arêa Leão A E and Cury A Deficiencias vitamin cas de cogumelos patogenicas Myc J th l et My l Appl 5 6 30 19 0

3 Baker R D The diagnosis of fungus disease by biopsy *J Chron Dis* 5 5 5 0 19 7

4 Beamer P R Smith E B and Barnett H L Histoplasmosis *J Pediat* 24 270 1944

5 Binford C H Histoplasmosis tissue reactions and morphologic variation of the fungus *Am J Clin Path* 25 25 36 1955

6 Blank F On the cell walls of Dimorphic fungi causing systemic infections *Canad J Microbiol* 1 1 5 1954

7 Bonorden R Growth of the yeast phase of *Histoplasma capsulatum* in a simplified fluid medium *Mycologia* 58 166 1956

8 Brown J H Cook K M Ney F C and Hatch T Influence of particle size upon the retention of particulate matter in the human lung *Am J Pub Health* 40 450 459 1950

9 Campbell C C Reverting *Histoplasma capsulatum* to the yeast phase *J Bact* 54 263 264 1947

10 Conant N F A cultural study of the life cycle of *Histoplasma capsulatum* Darling (1906) *J Bact* 41 563 578 1941

11 Cooke W B and Kobler P W The survival of *Histoplasma capsulatum* in water *Lloydia* 16 252 256 1953

12 Cozad G C and Furcolow M L Laboratory studies of *Histoplasma capsulatum* II Size of Spores *J Infect Dis* 92 72 81 1953

13 Cross F W The effect of hydrogen ion concentration on the yeast like phase of *Histoplasma capsulatum* (Darling) *Pub Health Rep* 63 739 746 1948

14 Darling S T A protozoan general infection producing pseudotubercles in lungs and focal necrosis in the liver spleen and lymph nodes *J A M A* 46 1283 1285 1906

15 Darling S T The morphology of the parasite (*Histoplasma capsulatum*) and the lesions of histoplasmosis a fatal disease of tropical America *J Exper Med* 11 515 531 1909

16 Da Rocha Lima H Histoplasmosis und epizootische lymphangitis *Arch Schiffs u Tropenhyg* 16 79 85 1912

17 DeMonbreun W A The cultivation and cultural characteristics of Darlings *Histoplasma capsulatum* *Am J Trop Med* 14 93 116 1934

18 DeMonbreun W A The dog as a natural host for *Histoplasma capsulatum* Report of a case of histoplasmosis in this animal *Am J Trop Med* 19 565 588 1939

19 Dowding E S The spores of *Histoplasma* *Canad J Research* 26 265 273 1948

20 Dowding E S Histoplasma and Brazilian Blastomyces *Mycologia* 4 668 679 1950

21 Drouhet E and Schwarz J Comparative studies with 18 strains of *Histoplasma* *J Lab & Clin Med* 47 128 139 1956

22 Drouhet E and Schwarz J Croissance et Morphogénèse d Histoplasma I Etude comparative des phases mycélienne et levure de 18 souche d *Histoplasma capsulatum* d origine Americaine et Africaine *Ann Inst Pasteur* 90 144 160 1956

23 Dubois A Janssens P G and Brutsaert P Un cas d histoplasmose africaine Avec une note mycologique sur *Histoplasma duboisii* V Sp par R Van breuseghem *Ann Soc Belge Med Trop* 3 569 584 1952

24 Dubois A and Vanbreuseghem R L histoplasmose africaine *Bull Acad Roy Méd Belgique* 17 551 564 1952

25 Dubois A and Vanbreuseghem R Etude expérimental d une souche Belge d *Histoplasma capsulatum* Comparison avec d autres souches et avec H duboisii *Ant van Leeuwenhoek* ? 103 11? 19.6

26 Emmons C W Isolation of *Histoplasma capsulatum* from soil *Pub Health Rep 64* 892 896 1949

27 Gordon Morris A The problem of a selective isolation medium for *Histoplasma capsulatum* Pub Health Monogr No 39 1956 pp 6 6

28 Grayston J T Altman P L and Gezad C C Experimental histoplasmosis in mice Pub Health Monogr No 39 19 6 pp 99 103

29 Haley L D Saprophytic form of *Histoplasma capsulatum in vivo* *Yale J Biol & Med* 4 381 383 1952

30 Hansmann C H and Schenken J R A unique infection in man caused by a new yeast like organism a pathogenic member of the genus *Sepedonium* *Am J Path 10* 731 ,38 1934

31 Helmbright A L and Larsh H W Size of the spores of *Histoplasma capsulatum* *Proc Soc Exper Biol & Med 81* 5 0 551 1952

3 Howell A Jr Studies on *Histoplasma capsulatum* and similar form species I Morphology and development *Mycologia 31* 191 216 1939

33 Howell A Jr Studies on *Histoplasma capsulatum* and similar form—species II Effect of temperature *Mycologia 3* 671 680 1940

34 Howell A Jr Studies on *Histoplasma capsulatum* and similar form species III Effect of hydrogen ion concentration *Mycologia 33* 103 117 1941

35 Howell A Jr The efficiency of methods for the isolation of *Histoplasma capsulatum* *Pub Health Rep 63* 173 178 1948

36 Kao C J and Schwarz J Heat resistance of seven strains of *Histoplasma* *J Infect Dis 99* 219 221 1956

37 Kligman A M and Baldridge C D Morphology of *Sporotrichum schenckii* and *Histoplasma capsulatum* in tissue *Arch Path 51* 67 571 19 1

38 Kurung Joseph M The isolation of *Histoplasma capsulatum* from sputum *Am Rev Tuberc 66* 5 8 587 19 ?

39 Kurung J M and Yegian D Medium for maintenance and conversion of *Histoplasma capsulatum* to yeast like phase *Am J Clin Path* 4 05 08 19 4

40 Larsh H W Conrad G C Hinton A and Furcolow M L The mouse as an aid in the isolation of *Histoplasma capsulatum* and the effect of adjuvants Public Health Monogr No 39 1956 pp 86 9?

41 Larsh H W Hinton A and Silberg S I Conversion and maintenance of *Histoplasma capsulatum* in tissue culture *Proc Soc Biol Med 93* 612-615 1956

4 Littman M L Liver spleen glucose blood agar for *Histoplasma capsulatum* and other fungi *Am J Clin Path* 5 1148 1159 19 5

43 Mariat F and Segretain S Etude mycologique D une Histoplasmose Spontanée Su Sin e Africa n (Cynocephalus babuin) *Ann L Institut Pasteur 91* 874 891 1956

44 McVickar D L Factors important for the growth of *Histoplasma capsulatum* in the yeast cell phase on peptone media I Blood and Blood Derivatives *J Bact* 6 137 143 1951

45 Menges R W Furcolow M L Larsh H W and Hinton A Laboratory studies on histoplasmosis I The effect of humidity and temperature on the growth of *Histoplasma capsulatum* *J Infect Dis 90* 67 70 195

46 Milne H A The morphology and cytochemistry of *H capsulatum* J M Lab Tech 14 112 163 195

47 Moore M *Posadasia pyriformis* and *P capsulata* two causative organisms of Darling's Histoplasmosis in the United States Ann Missouri Botan Garden 335 361 1935

48 Moore M A morphological and physiological study of two species of *Posadasia* *P capsulata* (Darling) Moore and *P pyriformis* Moore Ann Missouri Botan Garden 2 335 361 1935

49 Myers W F and Sherwood N I Experimental histoplasmosis in the grass frog *Rana pipiens* Bacterial Proc pp 114 115 1951

50 Negroni P Estudio micologico del primer caso Sud Americano de histoplasmosis Rev Instituto Bacteriologico (D N H) 9 239 291 1940

51 Negroni P Un nuevo caso de histoplasmosis Estudio micologico y terapeutico Rev argent dermatosif 30 212 219 1946

Nielsen C F and Evans R E A study of the sporulation of *Histoplasma capsulatum* J Bact 68 61 261 1954

53 Nickerson W J Physiological bases of morphogensis in animal disease fungi Tr N Y Acad Sci 13 140 145 1951

54 Pine L Studies on the growth of *Histoplasma capsulatum* I Growth of the yeast in liquid media J Bacteriol 68 671 679 1954

55 Pine L Studies on the growth of *Histoplasma capsulatum* II Growth of the yeast phase on agar media J Bact 70 375 381 1955

56 Pine L Studies on the growth of *Histoplasma capsulatum* III Effect of thiamin and other vitamins on the growth of the yeast and mycelial phases of *Histoplasma capsulatum* J Bact 74 239 245 1957

57 Pine L and Peacock C L Reaction of fumaric and with cysteine J Am Chem Soc 7 3153 195

58 Pine L and Peacock C L Studies on the growth of *Histoplasma capsulatum* IV Factors influencing conversion of the mycelial phase to the yeast phase J Bact 75 167 174 1958

59 Ribi F and Salvin S B Antigen from the yeast phase of *Histoplasma capsulatum* I Morphology of the cell as revealed by the electron microscope Exp Cell Research 10 394 404 1956

60 Ritter C Studies of the viability of *Histoplasma capsulatum* in tap water Am J Pub Health 44 1 99 1304 1954

61 Rowley D A Haberman R T and Emmons C W Histoplasmosis pathologic studies of fifty cats and fifty dogs from Loudoun County Virginia J Inf ct Dis 15 98 108 19

62 Rowley D A and Huber M Pathogenesis of experimental histoplasmosis in mice I Measurement of infecting dosages of the yeast phase of *Histoplasma capsulatum* J Infect Dis 96 1 4 183 19

63 Rowley D A and Huber M Growth of *Histoplasma capsulatum* in normal superinfected and immunized mice J Immunolo 7 15 93 1956

64 Rowley D A and Pine L Some nutritional factors influencing growth of yeast cells of *Histoplasma capsulatum* to mycelial colonies J Bact 67 59 00 19

65 Salvin S B Cultural studies on the yeast like phase of *Histoplasma capsulatum* Darling J Bact 54 6 660 1947

66 Salvin S B Cysteine and related compounds in the growth of the yeast like phase of *Histoplasma capsulatum* J Infect Dis 84 2 283 1949

6 Salvin S B Growth of the yeast like phase of *Histoplasma capsulatum* in a fluid medium *J Bact* 59 31 313 19 0

68 Scheff C S Biochemical and immunological properties of *Histoplasma capsulatum* No 6 0 *Yale J Biol & Med* 18 41 51 19 0

69 Scherr C H and Weaver R H The dimorphism phenomenon in yeasts *Bact Rev* 17 1 9 19 3

0 Scherr C H The influence of temperature and —SH groups on the growth of dimorphic pathogenic fungi *Bact Proc* 19 C p 8

71 Scherr C H Studies on the dimorphism of *Histoplasma capsulatum* I The roles of —SH group and incubator temperature *Exper Cell Research* 1 ?? 10? 19

7? Schubert M I Compounds of thiol acids with aldehydes *J Biol Chem* 114 311 3 0 193

73 Schwarz J Giant forms of *Histoplasma capsulatum* in tissue explant *Am J Clin Path* 3 808 903 19 3

74 Schwarz J and Drouhet E Morphologic features of an African strain of *Histoplasma* in Hamsters and Mice *Ar Path* 64 409 413 19

Titsworth E H and Grunberg E A medium for the growth and maintenance of the yeast like phase of *Histoplasma capsulatum* *Mycologia* 4 ?98 300 19 0

76 Umanzio C B Systemic Mycotic Infections V Further observations on *Histoplasma capsulatum* with brief notes on Darling's histoplasmosis *J Osteopathy* 58 10 ?3 19 1

Vanbreuseghem R Histoplasmosis and African histoplasmosis *Mycologia* 45 803 816 19 3

78 Vanbreuseghem R *Histoplasma duboisii* and large forms of *Histoplasma capsulatum* *Mycologia* 58 ?? 1 ?? 9 19

9 Vanbreuseghem R Tinea Capitis and African Histoplasmosis in the Belgian Congo *Tr N Y Acad Sc* 19 ?? ?31 19

80 Weed L A Large and small forms of *Blastomyces* and *Histoplasma* *Am J Clin Path* 1 ? 971 9?3 19 3

81 Zarafonetis C J D Dubos medium with albumin for yeast phase growth of *Histoplasma capsulatum* *Am J Clin Path* 911 91 19 ?

8? Zeidberg L D Ajello L Dillon A and Runyon L C Isolation of *Histoplasma capsulatum* from soil *Am J Pub Health* 4 930 93 19 ?

83 Zeidberg L D Ajello L and Webster R H Physical and chemical factors in relation to *Histoplasma capsulatum* in the soil *Science* 1 33 31 19

SAPROPHYTIC RESERVOIRS OF HISTOPLASMA

CHESTER W EMMONS

The sporadic occurrence of cases of histoplasmosis without evidence of contagion suggested early in the study of this mycosis that there might be a reservoir in nature from which man was infected Darling in reporting his discovery and original studies of histoplasmosis expressed the opinion that the microorganism (which he erroneously believed was a protozoan) had a saprophytic existence and he searched for it in stagnant water as well as in animals (4)

DeMonbreun in the second epochal advance in knowledge about histoplasmosis suggested also that Judging from its cultural characteristics the saprophytic form of Histoplasma capsulatum probably exists free in nature On the other hand it is possible that certain insects serve as carriers of the parasitic form of the fungus (5) The predictions of both men were confirmed in part in 1949 by the isolation of Histoplasma from soil and the demonstration of macroconidia of the saprophytic growth phase in the first two positive soil specimens (6)

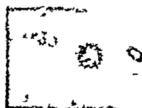


Fig 1 Macroconidium of Histoplasma from first positive soil sample

METHODS OF ISOLATION FROM SOIL

Methods used in isolating Histoplasma from soil are based upon the method used by Stewart and Meyer to isolate Coccidioides from soil (31) Several modifications of this method have been proposed The methods I have found most convenient and generally satisfactory have been described in the paper reporting the original isolation of Histoplasma from soil (6) and in a more recent report

of continued investigations of this occurrence (7) Briefly the method is as follows

At a time when the soil is dry collect samples in cotton stoppered 25 x 150 mm test tubes by scooping the soil up with the lip of the tube Select samples from soil under the edges of a chicken house or at any place indicated as the probable source of a patient s exposure If the specimens can not be processed immediately after collection store them in the cotton stoppered tubes at 1°C in order to permit further drying and to check growth and change in the microflora and microfauna

Transfer 10 to 15 ml of the soil to a second 25 x 150 mm tube add 0 8% NaCl solution to nearly fill the tube insert a sterile rubber stopper and shake vigorously for 15 20 seconds Allow the solids to settle for 10 15 minutes pipette 5 or 10 ml of the supernatant to a conical glass or small beaker and add for each 1 ml of supernatant 0 2 ml of antibiotic solution (2 mg streptomycin and 5 mg penicillin per 1 ml water) Inject 1 ml of this mixture intraperitoneally into each of 5 or 10 mice Occasional soil specimens contain bacteria pathogenic for mice and if inoculated mice appear ill on the following day inject an additional 0 25 ml of antibiotic solution intraperitoneally After 4 weeks kill the mice and make cultures of liver and spleen by spreading pieces of these organs with a stiff loop over the surface of Sabouraud s agar slants Incubate the cultures at 30°C or room temperature never at 37 C unless there are special reasons for isolating the fungus in the yeast phase In this case blood agar or another special medium must be substituted for Sabouraud s Cultures can usually be read in 10 days but should not be discarded as negative before 3 weeks

RELATIONSHIP OF ANIMALS TO THE SOIL RESERVOIR

The actual isolation of Histoplasma from soil (6) followed a systematic search which was begun in an area in northern Virginia where 4 fatal cases of histoplasmosis had been reported (25) where epidemiologic studies conducted over a period of years showed that 83% of the general population reacted to the histoplasmin intradermal test while 41 per cent had calcified pulmonary lesions (27) and where naturally acquired histoplasmosis was proved by isolation

of *H. capsulatum* in culture from about 3.5% of 2,149 Norway rats and from 7 other species of animals (11, 14). Later studies using the very sensitive technique of injecting ground mediastinal and hilar lymph nodes of healthy dogs and cats intraperitoneally into mice and culturing the livers and spleens of the experimentally infected mice at autopsy revealed that in this area 44 per cent of healthy dogs and cats had benign histoplasmosis (28, 13). These animals are not reservoirs or sources of Histoplasma in the sense that they transmit histoplasmosis directly to other animals or to man. The possibility that the rare case of fatal canine histoplasmosis (in which lesions of the intestinal mucosa are prominent) results in an effective seeding of soil can not be denied. Certainly large numbers of Histoplasma cells are excreted by these animals with intestinal lesions, but the ability of the delicate yeast-like cells of Histoplasma to survive biological competition in the feces and to germinate and establish themselves in soil where they fall has not been proved. It is not necessary to assume that such a seeding of soil is essential to the life cycle of Histoplasma nor to its persistence in an endemic area. Histoplasma grows sparsely on sterilized soil in the laboratory and maintains its fertility and viability longer on this medium than upon richer culture media ordinarily used. Moreover, actual isolations of literally hundreds of typical and virulent strains of Histoplasma from soil conclusively prove its saprophytic growth in soil or organic debris in nature under suitable conditions. The biological advantages of this ability to grow as a saprophyte will be discussed later in this chapter.

It is notable that Histoplasma was isolated from two other species of animals (the roof rat and spotted skunk) in southern Georgia where less than 3% of the population react to histoplasmin and where Histoplasma has not yet been isolated from soil, although its saprophytic growth in soil may be assumed here with almost as much confidence as in many other parts of the world where actual isolations have been reported (12). Dogs and cats could not be included conveniently among the species of animals examined in southern Georgia, although these species were found to be so frequently infected in Virginia. Where unwanted dogs or cats can be collected from rural owners and examined by the very productive

method mentioned above they provide a more direct sensitive and reliable index of the presence of Histoplasma in an area than dependence upon histoplasmin which may give nonspecific reactions in man and shows no correlation between dermal reactions and proved benign histoplasmosis in the dog (13) Mouse passage of dog lymph nodes has been more productive also than direct sampling of soil probably because the dog is a susceptible host and his close contact with soil makes him an efficient collector of Histoplasma from his environment

At the time systematic attempts to isolate Histoplasma from soil were begun the only guide to selection of probably productive sites was the proved occurrence of histoplasmosis in Norway rats trapped on certain farm premises and assumed to be infected from soil (11) Therefore soil samples were taken from rat burrows and runways found on these farms which were located in a rural county in northern Virginia The first positive samples were from mounds of clay soil at the entrance to a rat burrow under the edge of a chicken house (6) Macroconidia of *H capsulatum* were visually demonstrated in these first positive samples (Fig 1)

The studies of Zeidberg *et al* in Tennessee (31) and continued experience in northern Virginia (7 11) amply proved that it was the association with chicken droppings rather than the presence of rats which influenced the occurrence and distribution of Histoplasma in soil on the farm where the original isolations were made as well as on many other farm premises This association has been confirmed by many investigators (1 2 16 17 30) It is apparent also that it is not a host parasite relationship which is responsible for the association between Histoplasma and chickens No naturally infected chickens have been found and the chicken is not susceptible to progressive experimental infection although it can harbor injected spores of the fungus for varying periods of time It is probable that the single factor of the high body temperature of birds precludes the possibility of an avian reservoir There is no reason to believe that either an avian or animal reservoir of histoplasmosis is responsible for the distribution of Histoplasma in soil (2 7 21) On the contrary all the known evidence points to a saprophytic reservoir of the fungus in soil or organic debris from which both men and

animals (when exposed and susceptible) are infected

The saprophytic association with chickens need not be immediate in time or space. Histoplasma has been isolated from chicken coop litter deposited in small piles on an exposed hillside many weeks before (7, 10), from garden soil fertilized with chicken manure (20) and from underneath blue grass sod on the site of a chicken house torn down and removed three years previously (7). Follow up studies of these materials and sites to determine precisely how long Histoplasma can survive under such conditions have not yet been reported.

Not all chicken houses nor the soil under and around them have been found to be contaminated by Histoplasma, although similar buildings on neighboring farm premises may yield the fungus. Some attempts to isolate Histoplasma from commercial establishments producing eggs or fryers have been unsuccessful. Whether this is due to better sanitation in the average commercial chicken house, to inadequate sampling or to other unrecognized factors has not been determined. The isolations from inside chicken houses usually have been from old and poorly constructed buildings or coops with leaking roofs or open fronts which permit entry of rain so that the litter is frequently wet. However, Histoplasma may be and often is isolated from protected areas under the eaves and walls of well built chicken houses in good repair and from shaded and protected areas in chicken yards and runways.

SAPROPHYTIC OCCURRENCE NOT ASSOCIATED WITH CHICKENS

Although the association between the presence of Histoplasma in soil or organic debris and the presence of chickens has been shown in many studies, this association is neither necessary nor constant. Histoplasma was isolated repeatedly from the inner walls and the foundation ledge near the base of an old silo (unused for many years) in which birds had nested and from which members of a farm family were infected while cleaning the silo (21). In this admirable and fully documented study, serologic tests indicated histoplasmosis in only those members of the household who were exposed to dust

and debris from the silo the severity of the disease in those infected increased with the intensity of exposure and Histoplasma was isolated from only one of the patients The isolation of Histoplasma has been reported from the inside of hollow trees which were the probable sources of infection in children who had played in these places (16 17) from soil in a river bottom where fishermen had dug bait from the basement of a building in a city and from the walls of a storm cellar (16) Histoplasma was isolated many times and at all seasons of the year from the dirt floor of a meat house where home-cured bacon and hams were hung and where rat burrows were numerous in the dirt floor (7)

In an outbreak in Cincinnati involving several cases of pneumonitis in men engaged in cleaning out an old water tower in which pigeons had roosted and nested for many years the diagnosis of histoplasmosis was made in retrospect upon the basis of serologic and epidemiologic evidence and Histoplasma was isolated from the base of the tower (29) A similar epidemic with similar exposure was reported from Plattsburg New York (32) Unfortunately cryptococcosis was not considered in the differential diagnosis of the illnesses in either epidemic and Cryptococcus was not isolated from the organic material at the base of the tower although this fungus has since been shown to be very frequently associated with pigeon droppings and old pigeon nests (8 18 19 23)

HISTOPLASMA IN CAVES

In Peru Histoplasma has been isolated from the floor of a cave (Cueva de las Lechuzas near Tingo Maria) notorious as a source of infection known locally as Fiebre de Tingo Maria and now recognized as histoplasmosis (22) This cave two other caves in Peru one in Venezuela and one in Trinidad are inhabited by a nocturnal fruit eating bird *Steatornis caripensis* known locally as Gaucharo and as the oil bird It is prized as a source of oil obtained from its fat and residents of the area have frequented the cave to collect these birds The association between visits to Cueva de las Lechuzas and cases of Fiebre de Tingo Maria had been known to Peruvian physicians for some ten years before it was recognized

that this disease is histoplasmosis Histoplasma was isolated from a soil sample taken from the floor of a portion of the cave which harbors a concentration of *S caripensis* and the droppings of these birds are believed to support the growth of Histoplasma The possible role of bats in maintaining Histoplasma in this cave has not been determined

In Venezuela Histoplasma has been isolated from a cave in Estado Lara and an official warning was issued to speleologists and others that entrance into at least four Venezuelan caves and perhaps many *others is hazardous because of the danger of infection* by Histoplasma (3) *S caripensis* was not incriminated in these cases but it was suggested that bat guano may be the significant ecological factor and one Vampire bat (not infected with Histoplasma) was captured Histoplasma was isolated from dust of a cave in South Africa (26) Speleologists and persons removing bat guano from this and neighboring caves have acquired histoplasmosis This is in an area (the Transvaal) where histoplasmin sensitization in the general population is low Speleologists from the Cape have not encountered Histoplasma in caves in that area The ability of bat guano to support growth of Histoplasma is generally assumed It is probable that colonization by bats is the ecological factor responsible for the presence of Histoplasma in many caves Samples of bat guano from two caves and soil from five other limestone caves which did not harbor large colonies of bats were made in western Virginia and eastern West Virginia (7) Histoplasma was not isolated from any of these but this negative evidence of course may reflect only inadequate sampling or may indicate that not all caves which harbor bats are contaminated by Histoplasma just as some chicken houses in an endemic area are free of this fungus

A recent review of small epidemics of pneumonitis in persons entering caves in Mexico has been made by González Ochoa (15a) Epidemiologic evidence either in retrospect or in cases recently studied (2a) and laboratory evidence in the latter cases indicate that these illnesses were actually histoplasmosis They occurred for the most part in persons collecting bat guano for commercial use as fertilizer

HISTOPLASMA ASSOCIATED WITH THE HOUSE BAT

A recently studied family outbreak of histoplasmosis involving death of an infant and illness in several siblings appears to incriminate bats under circumstances which may be significant in interpreting the epidemiology of histoplasmosis in some urban areas (9). No chickens had been kept on these rural premises for many years although a small shed once used for chickens remained at the beginning of the study and was at that time used as a storage shed and dog house. Three soil samples taken from near this shed were positive but a much heavier concentration of Histoplasma was found around the residence of the family. This house was old, with loose siding and cornices and it harbored a large colony of the common brown bat *Eptesicus fuscus*. Histoplasma was isolated many times from soil samples taken adjacent to the foundation of the house on all four sides of the house and was uniformly absent or not demonstrable from soil samples taken at distances of several feet away from the foundation. Bat droppings were easily observed in soil samples taken adjacent to the foundation of the house. Bat colonization by *E. fuscus* (often called the "house bat") of old houses and shop buildings in some towns and cities is well known and should be investigated as a source of urban histoplasmosis.

GEOGRAPHIC DIFFERENCES IN RATES OF HISTOPLASMIN REACTIONS

Studies concerning the saprophytic occurrence in nature of Histoplasma can not be fully reported without a discussion of attempts which have been made to explain the geographic distribution of histoplasmin sensitivity on geologic or broad climatologic bases. It has been assumed that geographic areas of high prevalence of histoplasmin sensitivity coincide with the distribution of Histoplasma and histoplasmosis. While it is true that Histoplasma has been isolated from human cases, animals and soil most often in areas where a high percentage of the general population react to histoplasmin, the fungus and infections resulting from exposure to it occur also in areas where few persons react to the test, e.g., in southern Georgia and in South Africa as noted above. Moreover out

breaks of histoplasmosis and isolations of the fungus have been reported in small areas with localized high histoplasmin sensitivity rates in regions remote from eastern and central United States where rates have been shown to be unusually high Furthermore the maps showing histoplasmin rates while valuable and generally valid are not complete For example the tidewater areas of Virginia and Maryland although long known to harbor Histoplasma (25 27 10) have been omitted from earlier maps because the testing that was done involved only a few hundred people These maps tend to focus attention on a so-called endemic area an emphasis which may decrease the search for and recognition of cases of histoplasmosis remote from such areas

Regardless of these considerations attempts to explain the remarkable geographic differences in rates of histoplasmin sensitivity are highly commendable Zeidberg has pointed out a general coincidence between the distribution of red podzolic soils and that of histoplasmin sensitivity (33) It has been suggested also that the distribution of histoplasmosis lies within the great river basins (15) *that distribution is determined by the direction of prevailing winds* (15) and that sensitization to the fungus is more frequent in valleys than along adjacent ridges (35) These observations and theories may well have merit and deserve further investigation However they do not appear to fit the observed facts concerning the distribution of histoplasmosis outside the areas usually considered endemic and especially they minimize the importance of the highly localized distribution of Histoplasma on e g specific farm premises inside or outside areas of prevalent histoplasmin sensitivity

It is the general experience of investigators that Histoplasma can usually be isolated on a farm only within an area of a few square yards The association with chicken houses has been well documented earlier in the chapter but an additional instance studied over a period of many months might be cited On a large tobacco farm near Chesapeake Bay on Maryland s Eastern Shore Histoplasma could not be isolated from soil adjacent to any of many barns and outbuildings except one small poorly constructed chicken house Forty two of 142 samples taken from inside and under the eaves and walls of this shed yielded Histoplasma regularly at all

seasons of the year. In some collections as many as 70% of specimens were positive (7). Here as elsewhere Histoplasma can be found only in the superficial layers of soil, often in protected areas under the edge of the building but sometimes in dry soil with full exposure to sun. The correlations so far proposed between varying rates of histoplasmin reactors and geological and climatological factors do not explain the observed localized occurrence of Histoplasma in the United States nor elsewhere in the world.

Histoplasma shares with most of the fungi which cause subcutaneous and generalized infections a free living independent saprophytic existence in nature and inability to pass directly from an infected host to the latter's contacts. Only the saprophytic growth phases of these dimorphic fungi are effective in dissemination of the fungi and production of infection under natural conditions.

The biological advantages of the possession of a free living saprophytic growth phase are somewhat more apparent in the case of Histoplasma than for some of the other pathogens. The parasitic growth form is a delicate thin walled yeast which loses its viability within a few hours even in physiological salt solution. The macroconidia, microconidia and hyphal fragments of the saprophytic growth form, on the other hand, are resistant to drying, are produced in large numbers by the fungus and are well adapted to dissemination of viable infective elements.

REFERENCES

1. Ajello L. Occurrence of *Histoplasma capsulatum* and other human pathogenic molds in Panamanian soils. *Am J Trop Med & Hyg* 3 897 901 1954
2. Ajello L. Soil as natural reservoir for human pathogenic fungi. *Science* 123 876 879 1956
2a. Alarcón D G. Histoplasmosis pulmonar epidémica. *Gac Méd Méx* 87 4 750 1957
3. Campins H, Zubillaga Z C, Lopez I G and Dorante M. An epidemic of histoplasmosis in Venezuela. *Am J Trop Med & Hyg* 5 690 695 1956
4. Darling S T. Histoplasmosis: A fatal infectious disease resembling Kala azar found among natives of Tropical America. *Arch Int Med* 2 107 123 1908
5. DeMonbreun W A. The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum*. *Am J Trop Med* 14 93 125 1931
6. Emmons C W. Isolation of *Histoplasma capsulatum* from soil. *Pub Health Rep* 64 892 896 1949

7 Emmons C W The significance of saprophytism in the epidemiology of the mycoses *Tr New York Acad Sc* Ser II *17* 1 7 166 1954

8 Emmons C W Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (Columba Livia) *Am J Hyg* 62 227 232 1955

9 Emmons C W Association of bats with Histoplasmosis *Pub Health Rep* 73 590 595 1958

10 Emmons C W and Campbell C C Histoplasmosis in the District of Columbia Maryland and Virginia *Clin Proc Child Hosp* 13 225 235 1957

11 Emmons C W Bell J A and Olson B J Naturally occurring histoplasmosis in *Mus musculus* and *Rattus norvegicus* *Pub Health Rep* 62 1642 1646 1947

12 Emmons C W Morlan H B and Hill E L Histoplasmosis in rats and skunks in Georgia *Pub Health Rep* 64 1423 1430 1949

13 Emmons C W and Rowley D A Isolation of *Histoplasma capsulatum* from fresh and deep frozen peribronchial lymph nodes of dogs by mouse inoculation *J Lab & Clin Med* 45 303 307 1955

14 Emmons C W Rowley D A Olson B J Mattern C F T Bell J A Powell E and Marcey E A Histoplasmosis Proved occurrence of inapparent infection in dogs cats and other animals *Am J Hyg* 61 40-44 1955

15 Furcolow M L and Harr W H Air and water in the natural history of *Histoplasma capsulatum* Proc Conf on Histoplasmosis 1952 Pub Health Monogr No 39 1956 pp 282 288

15a González Ochoa A Histoplasmosis pulmonar aguda primaria *Gaceta Med Mex* 87 733 744 1957

16 Grayston J T and Furcolow M L The occurrence of histoplasmosis in epidemics—epidemiological studies *Am J Pub Health* 43 665 676 1953

17 Hazen E L Little G N and Mordaunt V Isolation of *Histoplasma capsulatum* from two natural sources in the Mohawk Valley one the probable point source of two cases of histoplasmosis *Am J Pub Health* 46 880 885 1956

18 Yamamoto S Ishida K and Sato A Isolation of *Cryptococcus neoformans* from pulmonary granuloma of a cat and from pigeon droppings *Jap J Vet Sci* 19 179 191 1957

19 Kao C J and Schwarz J The isolation of *Cryptococcus neoformans* from pigeon nests *Am J Clin Path* 27 652 663 1957

20 Kier J H Campbell C C Ajello L and Sutliff W D Acute bronchopneumonic histoplasmosis following exposure to infected garden soil *J A M A* 155 1230 1232 1954

21 Larsh H W Hinton A and Cozad C C Natural reservoir of *Histoplasma capsulatum* *Am J Hyg* 63 18 27 1956

22 Lazarus A S and Ajello L Aislamiento de *Histoplasma capsulatum* del suelo de una cueva en El Peru *Rev Med Exp Lima* 9 15 19

23 Littman M L *Cryptococcus neoformans* in pigeon excreta in New York City *Am J Hyg* 69 49 59 1959

24 Loosli C G Grayston J T Alexander F R and Tanzi F Epidemiological studies of pulmonary histoplasmosis in a farm family *Am J Hyg* 55 392-401 1952

25 McLeod J H Emmons C W Ross S and Burke F G Histoplasmosis *J Pediat* 28 2 [illegible] 1946

26 Murray J F, Lurie H I Kaye J Komins C Borok R and Way M Benign pulmonary histoplasmosis (Cave Disease) in South Africa *South African M J 31* 15 253 1957

27 Olson B J Bell J A and Emmons C W Studies on histoplasmosis in a rural community *Am J Pub Health 37* 441 449 1947

28 Rowley D A Haberman R T and Emmons C W Histoplasmosis pathologic studies of fifty cats and fifty dogs from Loudoun County Virginia *J Infect Dis* 95 98 108 1954

29 Sabin A B An epidemic of unusual granulomatous pneumonitis caused by Histoplasma Proc Conf on Histoplasmosis 1952 Publ c Health Monogr No 39 1956 pp 70 75

30 Silva M E Isolamento de *Histoplasma capsulatum* de solo em zona endemica de Calazar na Bahia Brasil *Bol* [illegible] *Cefalo Moniz* No 10 pp 1 1 1956

31 Stewart R A and Meyer K F Isolation of *Coccidioides immitis* (Stiles) from soil *Proc Soc Exper Biol Med* 9 93 938 1932

32 White F C and Hill H E Disseminated pulmonary calcification *Am Rev Tuberc* 6 1 16 1950

33 Zeidberg L D A theory to explain the geographic variations in the prevalence of histoplasmin sensitivity *Am J Trop Med & Hyg 3* 10 1 106 1954

34 Zeidberg L D Ajello L Dillon A and Runyon L C Isolation of *Histoplasma capsulatum* from soil *Am J Pub Health 42* 930 935 1952

35 Zeidberg L D Dillon A and Gass R S Some factors in the epidemiology of histoplasmin sensitivity in Williamson County Tennessee *Am J Pub Health 41* 80 89 1951

GEOGRAPHIC DISTRIBUTION OF HISTOPLASMA CAPSULATUM

LIBERO AJELLO

As a result of the discovery of the benign form of histoplasmosis by Christie and Petersen in 1945 (1) great interest has been aroused in this mycotic disease and its etiologic agent Histoplasma capsulatum The disease which formerly had been considered rare has come to be recognized as one that is widely prevalent and is a serious public health problem Consequently histoplasmosis and its causative fungus have been the subject of numerous investigations From clinical ecological epidemiological and skin test surveys carried out by many investigators throughout the world much has been learned about the geographic distribution of H capsulatum

Skin test surveys of histoplasmin sensitivity among the inhabitants of many regions of the world have furnished the most comprehensive information relative to geographic distribution Excellent reviews of these studies have been prepared by Manos *et al* (2) and by Edwards and Klaer (3) The distribution maps (see p 198) from these papers reveal that population centers with high histoplasmin reactivity exist in many areas of the world The existence of sensitivity in the inhabitants of a region is considered evidence that the sensitizing agent is also concentrated there since a positive histoplasmin reaction is generally indicative of specific infection by H capsulatum

Cross reactions to other fungus antigens are not known to occur except in those areas of the New World where Coccidioides immitis is endemic The coccidioidin positive individual often cross reacts with histoplasmin but usually his histoplasmin reaction is appreciably less intense

In order to avoid erroneous conclusions in delineating endemic areas skin test surveys must be conducted in such a manner that small islands of endemicity are not overlooked or that reactors who

may have acquired their sensitivity through travel or residence in other areas do not affect the validity of the results. For this reason population samples large enough to be statistically significant should be used and individuals who might have acquired their sensitivity elsewhere should be eliminated from the survey.

Unequivocal proof of the existence of H. capsulatum in a region is provided by recovery of the fungus from soil. Isolation studies leave no doubt that H. capsulatum exists as a free living microorganism in suitable habitats throughout the world. On this basis its endemicity has been established in North, Central and South America and in Africa and Asia (Table I).

TABLE I

RECORDED SOIL ISOLATIONS OF HISTOPLASMA CAPSULATUM THROUGHOUT THE WORLD

Region	*Reference*	*Region*	*Reference*
NORTH AMERICA		CENTRAL AMERICA	
United States		*Panama*	18
Alabama	4	SOUTH AMERICA	
Arkansas	5	*Brazil*	
Florida	6	Bahia	19
Illinois	5 7	*French Guiana*	20
Indiana	5	*Peru*	1
Iowa	5	*Trinidad*	22
Kansas	5	*Venezuela*	19 23
Kentucky	8	ASIA	
Maryland	9	*India*	
Minnesota	5	Poona	21
Missouri	5	AFRICA	
New York	10	*Union of South Africa*	
Ohio	5	Transvaal	25
Oklahoma	5	EUROPE	
Pennsylvania	9	*Italy*	
Tennessee	11 12 13	Lombardy†	
Texas	14		
Virginia	1		
West Virginia	16		
Wisconsin	5		
Mexico			
Tamaulipas	1		

For the present purposes H. duboisii is considered to be a synonym of H. capsulatum or at most a variety

† Personal communication Dr R. Ciferri

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In order to avoid erroneous conclusions in delineating endemic areas skin test surveys must be conducted in such a manner that small islands of endemicity are not overlooked or that reactors who

mated with bird droppings may provide conditions so favoring the development of H. capsulatum that the fungus can successfully compete with other soil microorganisms which ordinarily would outgrow and eliminate it.

There is no valid proof that chickens or other birds serve as carriers of this fungus (30), the experiments of Menges *et al.* (31) and Schwarz *et al.* (32) notwithstanding. These investigators demonstrated the survival of H. capsulatum yeast phase cells in test birds, but no evidence regarding the susceptibility of birds to infection was presented.

The detection and isolation of H. capsulatum in wild animals

TABLE III

HISTOPLASMA CAPSULATUM INFECTIONS IN DOMESTIC ANIMALS

Locality	*Species*	*Reference*
NORTH AMERICA		
United States		
Indiana	Dog	44
Iowa	Dog	44
Kansas	Dog	44
Kentucky	Dog	8
Michigan	Dog	44
Missouri	Dog	44
Ohio	Dog	44
Tennessee	Dog	44
Virginia	Dog	44
Washington	Dog	33
Kansas	Cat	4
Virginia	Cat	33
Tennessee	Horse	46 17
Missouri	Cow	48
SOUTH AMERICA		
Brazil	Dog	49
CENTRAL AMERICA		
Panama	Dog	50
CANADA		
Ontario	Dog	51
	Unsubstantiated Records	
Turkey	Cat	52
Hawaii	Cow	53
United States	Cat	54

It has been observed that H capsulatum is more apt to occur in avian habitats than in other locations This association was first detected by Zeidberg and Ajello (26) and has since been verified by many investigators (10 27 29) Although the basis for this relation ship remains unknown it has been hypothesized that soil contami

TABLE II

NATURALLY ACQUIRED HISTOPLASMA CAPSULATUM INFECTIONS IN WILD MAMMALS

Locality	*Species*	*Reference*
NORTH AMERICA		
United States		
Georgia	Didelphis virginiana—Opossum	37
Georgia	Mephitis mephitis—Striped skunk	37
Georgia	Procyon lotor—Raccoon	37
Georgia	Rattus norvegicus—Brown rat	33
Georgia	R rattus—Black rat	33
Georgia	Spilogale putorius—Spotted skunk	33
Georgia	Urocyon cinereoargenteus—Grey fox	37
Georgia	Vulpes fulva—Red fox	37
Kansas	P lotor	34
Kansas	M mephitis	34
Missouri	R norvegicus	34
Virginia	D virginiana	35
Virginia	Marmota monax—Woodchuck	35
Virginia	M Mephitis	35
Virginia	Mus musculus—House mouse	33
Virginia	R norvegicus	33
Virginia	U cinereoargenteus	33
SOUTH AMERICA		
Brazil		
Bahia	R norvegicus	3
AFRICA		
French Guinea	Cynocephalus babuin (Papio cynocephalus)—Yellow baboon	36
	Unsubstantiated Records	
SOUTH AMERICA		
Surinam	Large series of insects birds and mammals	38 40
EUROPE		
Italy	Mus rattus (R rattus)	41
ASIA		
India	Mouse (white laboratory)	42
NORTH AMERICA		
United States		
Illinois	Ferret	43

cats and horses in many states of the United States In addition canine infections have been diagnosed in Brazil Panama and Canada (Table III)

Finally human cases of histoplasmosis diagnosed on a histological basis or by culture furnish an insight into the distribution of H capsulatum Here the records must be interpreted judiciously for often an infection has been contracted in areas far from the place of diagnosis This type of difficulty was illustrated clearly by Symmers when he evaluated the reports of histoplasmosis diagnosed in Britain (55) Edwards and Klaer (3) compiled and cited references to all cases of human histoplasmosis diagnosed in the world with the exception of Canada and the U S A from 1906 1955 The interested reader is referred to those sources for pertinent data Records that have been reported since 1955 are listed in Table IV

DISCUSSION

Very intensive surveys of the prevalence of histoplasmin sensitivity have been carried out within the United States (2) Areas with histoplasmin sensitivity ranging from 70 to 90% among young adults with a life time of residency in the areas are found in the states of Alabama Arkansas Illinois Indiana Mississippi Missouri Ohio and Tennessee Centers with positive histoplasmin reactions of 10 to 40% occur over a wide area some of them are far from the centers of highest reactivity (see p 199)

Palmer Edwards and Allfather (63) hold that some of the histoplasmin sensitivity in the peripheral areas either represents cross reactions with coccidioidomycosis or non specific reactions with another undetermined fungus infection It is reasonable to assume that some of the histoplasmin sensitivity noted in California Arizona New Mexico and Western Texas represents cross reactivity with coccidioidomycosis But the possibility should be entertained that true histoplasmin sensitivity may also exist in those areas Favorable conditions for the survival and propagation of H capsulatum probably occur in the coccidioidomycosis endemic areas

There is evidence that the southeastern portion of the United States is not free of H capsulatum This fungus has been isolated

also serve to fix and reveal the presence of the fungus in a specific territory Wild animals rarely wander from their chosen territories and presumably become infected by H capsulatum spores present in their environment There is no evidence for the transmission of histoplasmosis from one animal to another Emmons (33) Menges (34) Silva (35) Marrit (36) Ajello (37) and their co workers have revealed that a large number and variety of mammals in several areas of the United States and at least one area in Brazil and one in Africa have naturally acquired H capsulatum infections (Table II)

Domestic animals particularly cats and dogs are also susceptible to infection by H capsulatum and records of such cases of histoplasmosis serve to verify the presence of the fungus in a given area Histoplasmosis has been diagnosed in a cow and among dogs

TABLE IV

HUMAN CASES OF HISTOPLASMOSIS DIAGNOSED BY CULTURE OR HISTOLOGICALLY OUTSIDE CANADA AND THE UNITED STATES

Country	Reference
NORTH AMERICA	
Mexico	
Tamaulipas	17
CENTRAL AMERICA	
Costa Rica‡	
CARIBBEAN AREA	
Puerto Rico‡	
SOUTH AMERICA	
Peru	56
EUROPE	
Italy	57
AFRICA	
Belgian Congo	58†
Rhodesia	59
Senegal	60
S West Africa	61
ASIA	
India	21† 62 66

Only cases not cited by Edwards and Klaer (3)

† For the present purposes *H duboisii* is considered to be a synonym of *H capsulatum* or at most a variety

‡ Personal communication Dr W D Sutliff

China are the only two major areas with no records of histoplasmosis As greater interest is shown in this mold and in histoplasmosis its presence undoubtedly will be established in those countries and in other localities

ADDENDUM Since submission of this manuscript a visit to India made possible the study of the human and soil isolates of *H duboisii (H capsulatum)* reported by Kalra Bocar and Rebello (21) Both of these isolates proved to be *Thielavia sepedonium* a common saprophytic soil fungus Thus there is no valid record for the isolation of *H capsulatum* from the soil in India

REFFRENCES

1 Chri tie A an l Petersen J C Puln onar calcif cat s in negative reactors to tuberculin 4 *J Pul Health 35* 1151 111 1945

2 Manos N F Ferebee S H an l Kerschl aum W F Geograj hic variation in tl e prevalence c f l istoj la min sensiti ity *Di Chest* 9 641-6C8 1956

3 Elwards P Q an l Klaer J H W rl l wi le geograj l c distribution of h stopla min sen itivity *An J Trop M d & Hyg* 5 35 2 1956

4 Hosty T S Ajello L Wallace C D H well J an l Moore J A small out break cf l i t pl mo *Am I e Tb and Pu lm Dis 78* 6 599 19 8

5 Furcol w M L an l Gray ton J F Occurrence of histoplasm sis n epi lem cs *Tr 49th An ial Meeti g Nat T berc A soc* 19 2 pj 83 91

6 Conant N F Pe s nal con municati n 19 8

7 Grayst n J T Loosli C C and Alexa ler E R The olation of Hi t j lasma cap i latum fro n l i an t use l l *Sc nce 114* 323 3 4 19 1

8 Meng s R W McClellan J I an l Au herman R J Canu e l istopla mo is an l Elastomycos s i Lexin ton Kentu cky *J 1 Fet M A 124* 202 207 19 4

9 Fm n on C W and Campbell C C H stopla mo is in the Di trict of Columbia Marylan l an l Vi ginia *Clin Proc Chil H sj 13* 29 235 19

10 Hazer F L Little C N an l Mo luant A Isc lation of H toj lasm a capsulatum from two nat iral s irces in the Mol awk Valley Or e of tl e j robable point sources cf two cases of hi topla m sis 4 *J Pub Health 46* 890 89 19 6

11 Ajello L an l Zeidberg L D Isolation of Histoplasma capsulatum an l Allescher a l li from so l *Sc ence 113* 662 663 19 1

12 Ajello L Soil as natu al reser o r for hu ian pathogenic fungi *Sc ence 123* 8 6 8 9 19 6

13 K er J H Campbell C C Ajello L and Sutliff W D Acute bron l o-pneu non c histopla mos s f llow ng exposure to infected garden soil *J A M A 155* 12 0 123 19 4

14 Larsh H W H nten A and Co a l G C Nat iral reservo r of Histoplasm a caps ilatum *Am J Hyg 63* 18 27 19 6

15 Emmons C W I olat n of Histoplasma capsulatum from so l *Pub He lth Rep 64* 89 896 1949

16 Emmons C W Personal commi n cat on 19 8

from soils collected in Alabama (4) Florida (6) Maryland (9) Virginia (15) and West Virginia (16) In addition many wild and domestic animals in Georgia and Virginia have yielded cultures of H capsulatum (33 27 11) Campbell (61) in an admirable epidemiological study carried out in the District of Columbia area has stated rightly that histoplasmin sensitivity distribution maps are not entirely accurate

This inaccuracy stems in part from statistically invalid population samples used in the surveys For example only 1 520 life time residents were skin tested in Alabama 1 383 in Georgia 850 in Maryland 156 in Virginia and 58 in the District of Columbia (?) These areas have respective populations of 3 061 743 3,444 578 2 343 001 3 318 680 and 802 178

Palmer *et al* (63) have postulated a fungus disease other than histoplasmosis as responsible for the histoplasmin sensitivity noted in eastern Texas and in parts of Kansas Louisiana and Oklahoma This observation was made despite the fact that there is no clinical histological or mycologic evidence for the existence in those areas of an unusual prevalence of mycotic diseases that might cross react immunologically with histoplasmosis Since H capsulatum has been recovered from soils collected in Kansas (5) Oklahoma (5) and Texas (14) it seems logical to conclude that the sensitivity reactions elicited in the sample population represent specific rather than non specific reactions

H capsulatum endemic areas in the United States are not as sharply circumscribed as those of Coccidioides immitis and many foci are scattered in suitable areas far from the principle endemic regions (10 65)

It is unrealistic to conclude that histoplasmosis is primarily a disease of the Americas (3) Well authenticated indigenous cases have been reported from Europe Africa Asia and Australia (3) In addition *H capsulatum has been recovered from soil in India and* in the Union of South Africa (24 25) At present all that can be said is that H capsulatum apparently is more prevalent in some areas of the United States and Mexico than elsewhere in the world

What emerges from the data presented is the clear cut fact that H capsulatum is endemic in most areas of the world Russia and

von Suriname ? Vogel als Trager von I iste plasma artigen Mikroorganismen *Ztschr Hyg 135* 358 310 19?

39 Collier W A and I Winckel W F F 6 Histoplasmose bei Saugetieren in Suri name *Anthonie van Leeuwenhoek 18* 319 3 (19 2

40 Wildervanck A Collier W A and Winckel W F F Two cases of histoplas mosis on farms near Paramaribo (Surinam) investigations into the epidemi ology of the disease *Docum Med Geograph et Tropica* 5 108 115 19 3

41 Sangiorgi G Blastomicosi spontanea nei Muridi *Patholog ca 14* 493-49? 19?2

4 Shortt H E The pathogenicity of insect flagellates to vertebrates with special reference to Herpetemonas ctenocephali Fantham *Indian J M Res 10* 908 933 19?3

43 Levine N D Dunlap G L and Graham R An intracellular parasite encoun tered in a ferret *Co nell I et* 28 249 51 1938

44 Menges R W Canine histoplasmosis *J Am I et M A 119* 411-415 1951

45 Menges R W Furcolow M L and Haberman R T An outbreak of histo plasmosis involving animals and man *Am J I et Res 15* 5 0 5?1 1954

46 Richman H Histoplasmos s in a colt *North Am I et* 9 710 1918

47 Randall C C and McVickar D L Histoplasma capsulatum in ti sue culture *Proc Soc Exper Biol & Med* 77 150 153 1951

48 Menges R W and Kintner L D Bovine histoplasmosis *North A n I et 3* 69? 69 19 1

49 Para M H stoplasmosis in Brazil *Am J Trop Med* ?6 ?73 29? 1946

50 Tomlinson W J and Grocott R G Canine histoplasmosis A pathologic study of the three reported cases and the f r t case found in the Canal Zone *Am J Cl n Path 15* 501 507 1915

51 Fish N A Schroder J D and Fischer J B A laboratory report on a case of canine histoplasmos s in Ontario *C ad M A J 74* 731 73 1956

5 Akun R Fine Katzen Histoplasmose in der Turkei *Askeri I eteriner Dergisi 17* 349 351 19 0

53 Adler H E Generalized infection with a yeast like fungus in a range bull *No th Am I et 31* 4 7-4 8 1950

54 Meleney H E Toxoplasmosis mistaken for histoplasm o is *Am J Trop Med 25* 163 1945

55 Symmers W St C H stoplasmosis contracted in Britain A case of histoplasmic lymphadenit s following clinical recovery from sarcoidosis *Brit M J* 786 790 19 6

56 Arellano R C Z and Galvez J B El agente etiologico de la f ebre de Tingo Maria *Ann Fac Med 38* 109? 1098 1955

57 Sotgiu G and Corelli G Micos rari O servazione dei primi due case di isto plasmose in Italia e di un caso di coccid o domicos s *Bull sc med 1* 7 85 9? 19 5

58 Duboi A Janssens P G Dri t ert P and Vanbreuseghem R Un cas d his toplasmose africai e avec u e note mycolog que sur H stoplasma dubo s i n sp *Ann Soc Pelge Med T op 32* 569 584 19 ?

59 S m on F W and Ba netson J H stoplasmos s Report of a case *J Path & Bact 54* 99 305 194

60 Friess and Delvoye A p opos d un aspect chirurg cal d une mycose rare *Cah Med de l Union Franco e Alg r* 419 4 5 1947

17 Gonzales Ochoa A Personal communication 1957
18 Ajello L Occurrence of Histoplasma capsulatum and other pathogenic molds in Panamanian soil *Am J Trop Med & Hyg* 3 897 904 1954
19 Silva M E Isolamento de Histoplasma capsulatum do solo em zona endemica de Calazar na Bahia Brasil *Bol Fundacao Goncalo Moni* No 10 November pp 1 15 1956
20 Floch H and Andre J L histoplasmose de S T Darling III Isolement de *H* capsulatum a partir du sol en Guyane Française *Arch Inst Pasteur Guyane Française et L Inini 16* 1 11 July 1955
21 Lazarus A S and Ajello L Aislamiento de Histoplasma capsulatum del suelo de una cueva en el Peru *Rev med exp Lima* 9 5 15 1955
22 Ajello L and Downs W Unpublished data
23 Campins H Zubillaga C Z Gomez I L and Dorante M Estudio de una epidemia de histoplasmoses en la estado Lara Venezuela *Gac med Caracas* 62 85 109 1955
24 Kalra S L Borcar M D S and Rebello E R F Histoplasmosis isolation of the fungus from soil and man *Indian J M Sc 11* 496 498 1957
25 Murray J F Lurie H I Kaye J Komins C Borok R and Way M Benign pulmonary histoplasmosis (cave disease) in South Africa *South African M J 31* 245 253 1957
26 Zeidberg L D Ajello L Dillon A and Runyon L C The isolation of Histoplasma capsulatum from soil *Am J Pub Health 42* 930 935 1952
27 Emmons C W The significance of saprophytism in the epidemiology of the mycoses *Tr New York Acad Sc 17* 157 166 1954
28 Campbell C C A family outbreak of histoplasmosis II Epidemologic studies *J Lab & Clin Med 50* 841 848 1957
29 Grayston J T and Furcolow M L The occurrence of histoplasmosis in epidemics Epidemiological studies *Am J Pub Health 43* 665 676 1953
30 Zeidberg L D and Ajello L Environmental factors influencing the occurrence of Histoplasma capsulatum and Microsporum gypseum in soil *J Bact 68* 156 159 1954
31 Menges R W and Haberman R T Experimental avian histoplasmosis *Am J Vet Res 16* 314 320 1955
32 Schwarz J Baum G L Wang C J K Bingham E L and Rubel H Successful infection of pigeons and chickens with Histoplasma capsulatum *Mycopath et Mycologia Applicata 8* 189 193 1957
33 Emmons C W Rowley D A Olson B J Mattern C F T Bell J A Powell E and Marcey E A Histoplasmosis Proved occurrence of inapparent infection in dogs cats and other animals *Am J Hyg 61* 40 44 1955
34 Menges R W Furcolow M L and Hinton A The role of animals in the epidemiology of histoplasmosis *Am J Hyg 59* 113 118 1954
35 Silva M E and Paula L A Infeccao natural de ratos pelo Histoplasma capsulatum na cidade do Salvador Bahia *Bol Fundacao Goncalo Moniz* No 9 January pp 1 17 1956
36 Mariat F and Segretain G Etude mycologique d une histoplasmose spontanee du singe Africain (Cynocephalus babuin) *Ann Inst Pasteur 91* 874 891 1956
37 Ajello L and Richards C S Unpublished data
38 Collier W A and Winckel W E F Beitrag zur geographischen Pathologie

RESISTANCE OF ANIMALS AND MAN TO HISTOPLASMOSIS

S. B. Saslaw

Resistance to infection with Histoplasma capsulatum can be induced in experimental animals by sublethal infection or by vaccination with killed yeast cells or fractions thereof. Pathologic changes attributable to the disease are not as extensive in resistant as in control animals. Immunized animals when challenged with lethal doses show far lower death rates than nonimmunized controls. Finally, multiplication of the pathogen is actually inhibited within the tissues of immunized animals. However, the basis of this increased resistance is not yet known.

Data are not available which indicate why H. capsulatum is virulent. As far as is known, cells of the tissue or yeast phase do not produce extracellular enzymes which are injurious to the host. They do not produce demonstrable exotoxins. They do not seem to possess a capsular substance which can inhibit phagocytosis (17). An antigenic factor has not been demonstrated which is correlated with virulence. An endotoxin has been demonstrated (19) but its role in the infectious process has not been investigated.

INCREASED RESISTANCE FOLLOWING SUBLETHAL INFECTION

Increased resistance to reinfection has been demonstrated in mice, guinea pigs, and dogs (3, 22, 31). In addition, infection was modified in the uninoculated eyes of rabbits whose other eyes had been inoculated with the mycelial phase 6-14 weeks earlier (2).

Resistance was demonstrated in immature (35 day old) male mice which had originally been infected with a sublethal dose and later subjected to an intracerebral challenge lethal for normal mice (22). Such mice had a striking decrease in death rate. For example, where 92% of control mice died, only 22% of the superinfected

61 Duncan J T A unique form of Histoplasma *Tr Roy Soc Trop Med & Hyg* *40* 361 365 1946 47
62 Zaoli G L istoplasmosi oro faringea *Arch ital Otologia Rinologia e Laringologia* *68* 374 403 1957
63 Palmer C E Edwards P Q and Allfather W E Characteristics of skin reactions to coccidioidin and histoplasmin with evidence of an unidentified source of sensitization *Am J Hyg* *66* 196 213 1957
64 Campbell C C A family outbreak of histoplasmosis II Epidemiologic studies *J Lab & Clin Med* *50* 811 818 1957
65 Murphy R J Peck W M and Vincent B Preliminary report of histoplasmin and other antigen sensitivity in North Carolina *Am J Pub Health* *41* 1521 1525 1951
66 Sen Gupta P C Rao A Banerjee A K Chakraborty A N and Ray H N Histoplasmosis *Bull Calcutta School Trop Med* *5* 54 56 1957

INCREASED RESISTANCE AFTER VACCINATION WITH KILLED WHOLE CELLS

Resistance to lethal challenge has been produced by injection of killed cells of the yeast phase or fractions thereof (11 20 21) In one laboratory cells of the yeast phase were grown in liquid medium then killed by exposure to 0 5% formalin at room temperature for 72 hours or by exposure to 1 5000 merthiolate at 37 C for 5 days Mice immunized intraperitoneally with such cells dried in acetone at −10°C or *in vacuo* resisted a lethal intracerebral challenge 2 weeks later (20) This work was later confirmed (14) Mice injected with formalin killed suspensions of yeast phase organisms once weekly for 3 weeks resisted lethal intravenous challenge Also mice injected with heat killed cells resisted lethal intraperitoneal challenge with yeast cells in 5% mucin There is one report of failure to protect animals with killed yeast phase organisms (18)

Although vaccinated mice tended to survive lethal intracerebral or intravenous challenge cultures of some of the tissues of these animals were positive Quantitative cultural studies showed that immunization lowered the number of yeast cells in the host tissues but did not eliminate the pathogen completely This effect was apparently sufficient to prevent death in the protected mouse subjected to lethal challenge Live cells of the yeast phase were present in the resistant mouse for long periods after challenge although death did not ensue and histologic study indicated a tendency for the disease to recede The mouse resistant to H capsulatum may react to the pathogen as a susceptible individual reacts to BCG in tuberculosis resistance in that growth of the pathogen occurs within the host tissues but to a limited extent

Studies of the pathology of infection after immunization have confirmed and extended the findings from the foregoing mortality and cultural studies (7) Protection of the brain against intracerebral infection was striking in vaccinated mice as compared to control animals in which extensive meningitis and parenchymal abscesses frequently occurred The amount of liver and spleen disease in the same animals was however only slightly less than in the controls Cultural and histologic examination revealed that immunization procedures did not [illegible] of the fungus from the

mice succumbed to challenge Neither size of the sublethal infection nor route of injection seemed of importance in the induction of resistance although the rate of development of this resistance did vary with the size of the immunizing dose In immature male mice inoculated intraperitoneally with 10^6 cells of the yeast phase about 3 days were required for the resistance to become apparent (22) *Resistance to lethal intravenous superchallenge has also been* demonstrated (13 18)

An immunizing infection does not entirely eliminate the pathogenic fungus from the host tissues In superinfected animals the fungus did not multiply or develop as much as it did in control animals with the restriction of growth noted especially in the spleen liver and site of inoculation Twenty four hours after challenge counts of the pathogen (obtained with the aid of P_3 labelled yeast cells) were found to be significantly lower in the tissues of reinfected animals than in those of controls (8)

Evidence suggests that man also is more resistant to reinfection Of 13 epidemics of histoplasmosis described (6) only three occurred in the area of greatest endemicity of histoplasmosis as shown by histoplasmin sensitivity tests Some of the epidemics appeared in areas of low histoplasmin sensitivity i e areas where less than 10% of the adults reacted to histoplasmin Also all the soldiers involved in the epidemics at Camp Crowder Missouri and Camp Gruber Oklahoma *were from states with a low ratio of reaction* to histoplasmin The probability of these patients having been exposed to previous infection therefore is low Accordingly when they were exposed to spores of the fungus in many cases severe disease followed

Children frequently have been involved in epidemics occurring in areas with a relatively high histoplasmin sensitivity The number of histoplasmin reactors in children have been found to be lower than in adults in the same area Involvement of children in many of the epidemics *would therefore suggest that the stricken individuals* did not have previous contact with the fungus In one epidemic at Kansas City Missouri skin reactions of three children and one adult converted from negative to positive (6)

A cell suspension of the yeast phase was killed either with three volumes of ethyl ether or 1 5000 merthiolate (29) The cells were subsequently harvested washed by centrifugation in distilled water and exposed to vibration in a Mickle tissue disintegrator Subsequent washings in distilled water produced two fractions the cell wall fraction and the protoplasmic fraction Previous examination of the intact cell by means of electron microscopy had not indicated the presence of a capsule (17) The morphology of cell wall and protoplasmic fractions under the electron microscope was so strikingly dissimilar that differentiation was simple The protoplasm of merthiolate killed cells was released in a fine uniform dispersed state which condition permits ready separation of cell walls by centrifugation and filtration In contrast the protoplasm of ether killed cells tended to contain more insoluble nondispersible gran

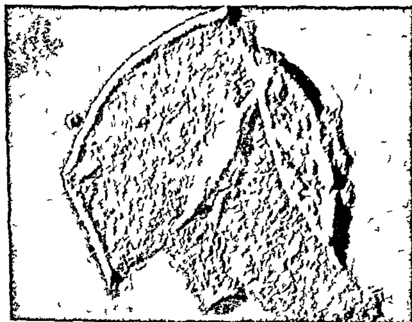


Fig 1 Cell wall of H capsulatum (x85 000 reduced about 5% from original illustration) Portion on left shows the granular outer surface section on lower right illustrates the fibrillar inner surface (Photo obtained through the cooperation of Dr E Ribi)

site of the inoculum possibly because of its predilection for growing in cells of the reticuloendothelial system

Living cells of the yeast phase are more effective immunizing agents than killed cells Immunization of mice with formalin killed cells of the yeast phase produces less resistance than an equivalent number of living fungus cells The apparently higher immunogenicity of living cells has not been satisfactorily explained but the basis of this phenomenon may again be analogous to that of immunization against tuberculosis with the BCG strain Several possible reasons for the increased immunogenic activity of living cells of H capsulatum may be presented

(a) Following introduction of fungus cells into the tissue a brief period of multiplication ensues in which the immunogenic material increases to a point where an effective immune response is produced The number of microorganisms living or dead originally introduced was too small to produce this immune response Experimental data have been obtained which indicate the occurrence of this limited multiplication of the fungus in non immune mice before a decrease in numbers starts (21)

(b) The living fungus cells although not actually increasing in number produce in the host tissue increased quantities of the specific immunogenic substance which is also present in the killed cells

(c) The process of killing the fungus cells partly destroys or alters the immunogenic substance

(d) Living microorganisms elaborate some complex in the host tissues which is not produced in the in vitro growth of cells of the yeast phase and which enhances immunity

INCREASED RESISTANCE TO CELL FRACTIONS

After the demonstration that killed cells of the yeast phase could induce increased resistance to invasion of the fungus investigations were initiated to isolate and identify purified active components which from a practical standpoint could be used in humans with greater safety Several techniques have been used to obtain such fractions

cipitin tests was crude filtrate of the broth in which either the mycelial or yeast phase was grown This filtrate therefore may and probably does contain several antigenic substances each of which may react with its own antibody in the serum

Similarly complement fixing antibodies do not indicate the resistance of the animal to infection Guinea pigs whose sera contained high titers of complement fixing antibodies with whole killed yeast cells as antigens did not necessarily show increased resistance as determined by quantitative counts of the fungus cells in the spleen liver kidney brain or lungs On the other hand some guinea pigs whose sera had complement fixing antibodies in low dilutions showed striking resistance to infection

Attempts have been made to detect neutralizing antibodies in patients with histoplasmosis (27) The technique consisted of incubating an LD_{100} or LD_0 of 48 hour old cells of the yeast phase with an equal volume of human antiserum at 37 C for 1 to 2 hours and then injecting the mixture intracerebrally into 35-day-old male mice After injection of the LD_{100} mortality was recorded for 28 days Seven or 14 days after injection of the LD_0 the tissues of the mice were cultured quantitatively Some human sera showed strong neutralizing properties Sera from one patient (F B) (16) for example showed a significant level of neutralizing antibodies after his recovery from a brief episode of histoplasmosis A correlation however could not be found between the presence of neutralizing antibodies and the course of the disease recoverability of the fungus from the host or presence of other antibodies Neutralizing antibodies were not detected in sera from experimentally infected rabbits guinea pigs or mice

Attempts to demonstrate participation of circulating antibody in acquired resistance also included experiments wherein the ability of whole blood or serum from resistant guinea pigs to inhibit growth of the yeast phase in vitro was measured (27) Yeast cells were incubated with immune serum at 37 C for 1 to 2 hours and then plated on Sabouraud s agar for quantitative counts The resulting growth was compared with that of cells incubated in the presence of control serum No definite indication could be obtained that the serum of resistant guinea pigs appreciably inhibited growth of the

ules the larger of which were separated from cell walls only with difficulty Extreme difficulty was encountered in attempts to separate the two fractions in formalin killed organisms

The protoplasmic fraction had little or no protective powers but mice were protected against lethal intracerebral or intraperitoneal challenge by prior intraperitoneal injection of the cell wall fraction (29) This fraction was effective in quantities of about 0 5 to 5 0 mg of dried material per mouse while the optimum dose for whole cells was about 5 mg per mouse Again the cell wall fraction apparently induced resistance sufficient to inhibit growth of the fungus and protect the life of the mouse but not to prevent growth of the pathogen completely (Fig 1)

Guinea pigs were also injected intraperitoneally with whole cells the cell wall fraction or the protoplasmic fraction When the animals were subsequently challenged with a sublethal dose of live cells the guinea pigs that had received the cell wall fraction had the most resistance in that fewer fungus cells were found in their tissues Complement fixing antibodies developed in response to all three types of antigenic fractions

NATURE OF ACQUIRED RESISTANCE

Although immunity or resistance to infection with H capsulatum is distinguished by the increased ability of the host to inhibit multiplication of the fungus the mechanism of this fungistatic response is not known Since the spread of many infectious processes seems to be limited by specific circulating antibodies attempts have been made to correlate precipitating complement fixing or neutralizing antibodies with increased resistance Little if any success has resulted thus far

No apparent correlation for example exists between the development of precipitins and the resistance to dissemination of disease (28) Where one patient might have high precipitin titers and a mild brief illness another patient might have high precipitin titers and a prolonged severe illness Precipitins appear early in the course of the disease but do not necessarily indicate the resistance of the patient to the infection The soluble antigen used in the pre

istic x-ray changes plus a positive skin test for delayed type of hypersensitivity indicate that this patient had had histoplasmosis but of a nonsystemic nonfatal nature. This delayed hypersensitivity to histoplasmin could be due (a) to the small amount of gamma globulin known to be present in hypogammaglobulinemic patients (b) to a stage in immunity wherein conventional antibodies have not yet been released into the circulating fluids or (c) to a qualitatively different immunologic response (25)

The theory that antibody may be involved in acquired resistance should not be completely abandoned. The techniques available for antibody determination may be inadequate for detection of small amounts of particular types of antibody. Some evidence exists for example that antibodies to different fractions of the yeast cell of H capsulatum appear at different times during the course of infection (10). Also different individuals may respond to a different degree to the various antigenic components of the fungus. It may therefore be extremely difficult by existing serologic techniques to detect small amounts of the antibody associated with immunity. This antibody may also be different from the conventional kind circulating in the gamma globulin and may exist in some other part of the serum globulin. Alternatively the antibody may not be in the circulation but may exist intracellularly such as within leucocytes. It should be emphasized however that the acquired resistance is specific and that it develops along patterns and at times similar to those seen in diseases wherein circulating antibody is known to be the effective agent. However the very fact that a disease such as histoplasmosis may be chronic suggests that resistance based on humoral antibodies may be relatively inadequate

The resistance to infection may be based on some mechanism involving cellular phagocytosis. Washed leucocytes from peritoneal cavities of immunized guinea pigs have been reported to phagocytize more fungus cells than leucocytes from normal animals. Similar quantities of mononuclear cells from resistant and control animals were exposed in Tyrode s solution to a given number of washed cells of the yeast phase in a given period of time and the number of phagocytized cells were compared (24)

fungus in vitro The results however are complicated by uncontrollable factors and may be misleading Accurate counting of the exact numbers of viable yeast cells is very difficult Also the possible effect of normal serum on growth and multiplication of H capsulatum has not been examined sufficiently

The passive serum protection test may indicate what part humoral antibody has in acquired resistance The test indicates whether serum from a resistant animal contains a specific substance or antibody which on transfer to a homologous nonimmune animal induces specific increased resistance in that animal The application of this test to mice or guinea pigs before during and after challenge produced no determinable change in the course of infection Promotion of phagocytosis by antibody is also of doubtful significance since cells of H capsulatum are readily and rapidly phagocytized by leucocytes of the normal non resistant animal

Of possible importance in the relationship of antibody to acquired resistance is the reaction to infection with H capsulatum of agammaglobulinemic individuals who have a strikingly poor capacity to form gamma globulin Such individuals are especially susceptible to those bacterial diseases in which it is known that acquired immunity depends on circulating antibody They also have a scarcity of plasma cells both before and after intensive vaccination (5) To the writer s knowledge no such agammaglobulinemic individuals are known who have shown an increased susceptibility to histoplasmosis or similar systemic fungus diseases Also the occurrence of clinical tuberculosis in agammaglobulinemic patients has not yet been noted (16) Presumably therefore the circulating antibody in the gamma globulin plays a minor role in resistance to histoplasmosis Alternatively the possibility exists that the tiny amount of antibody in the agammaglobulinemic person is sufficient to induce increased resistance to the disease

Idiopathic hypogammaglobulinemia was recently described in an adult male who was found to be sensitive to skin tests with histoplasmin but not to tests with blastomycin coccidioidin and old tuberculin (32) On three occasions complement fixing antibodies to H capsulatum could not be detected Also numerous attempts to culture the fungus were unsuccessful Nevertheless character

of delayed shock were still absent When the interval was increased to 12 days or more both resistance and delayed shock were present Delayed shock therefore can alter or nullify the beneficial effects of acquired resistance

The influence of delayed hypersensitivity on the spread of histoplasmosis within a host was further examined (26) according to the technique of Chase for passive transfer of delayed type of hyper sensitivity (1 11) Mice were injected with either live or killed cells of the yeast phase Two weeks later these mice which possessed both delayed sensitivity and acquired resistance were killed and bled Washed leucocytes from the blood of resistant sensitized mice were injected intraperitoneally into normal mice with the assumption that hypersensitivity of the delayed type was being transferred Two days later these recipient mice were challenged with a sublethal dose and subsequently tissues were cultured quantitatively Tissues such as the spleen and liver of the recipients contained greater numbers of fungal cells than those of the controls Presumably the introduction of white blood cells from a sensitized donor not only failed to increase the resistance of the host but actually seemed to lower the resistance and thereby make the recipient s tissues more favorable for multiplication of the fungus

NATURAL RFSISTANCF

Some experimental and epidemiologic work has been reported on the innate resistance of animals and man to infection with H capsulatum For example variation in susceptibility to intracere bral challenge was noted in a comparison of 7 strains of mice of which male dba line 1 mice were among the most susceptible (9) White Swiss male mice up to 15 weeks of age were more susceptible to experimental infection than mice 17 weeks of age or older (30) This difference may be related to the development of sexual maturity In immature mice (35 days of age) (20) no difference in susceptibility was noted between the two sexes Between 8 and 14 weeks of age females appeared to be more resistant than males (30)

Differences in incidence of histoplasmosis which have also been recorded among human populations (12 15) may be reflec

Cellular aspects of resistance were also examined in in vivo experiments using P_{32} labeled H capsulatum and in in vitro experiments with macrophages from peritoneal exudate (8) After injection of similar quantities of P_{32} labelled organisms into immunized chronically infected and normal mice comparison was made of the specific radioactivity of the spleens from the three groups After 24 hours lower counts were observed in the tissues of resistant animals These data indicate an increased rate of destruction of phagocytized fungus cells by macrophages of resistant animals Peritoneal phagocytes from normal and resistant mice adjusted to equal concentrations on the walls of flasks were exposed to a suspension of cells of the yeast phase At intervals thereafter counts were made of the radioactivity of washed fungus cells from the supernatant medium The results suggested that macrophages from immunized animals phagocytize more fungus cells than macrophages from control animals

Evidence therefore exists that increased resistance of an animal to histoplasmosis may result from increased capacity of its macrophages to inhibit growth of the fungus Data are however still scanty and more details are needed before definite conclusions can be drawn

HYPERSENSITIVITY AND RESISTANCE

Increased resistance to reinfection was demonstrated when mice originally infected with sublethal doses were challenged about 2 weeks later with a lethal dose via the intracerebral intraperitoneal or intravenous routes During the first 48 hours after such intraperitoneal challenge with 10^9 cells in 5% mucin many mice died (23) These deaths occurring too early to be due to infection and absent from control mice not previously inoculated were attributed to delayed or tuberculin type of shock Apparently during the 14 day interval between immunizing and challenging injections the animals developed susceptibility to death due to hypersensitivity as well as resistance to subsequent challenge When the interval between the two infections was decreased to a single day neither resistance nor shock was observed When the interval was 5 to 7 days resistance to lethal challenge was striking but signs

sis. I Metl d for { roduction of histop[la m sis in dogs *Proc Soc Exper Biol & Med 84* 1 51 1953
4 Furcolow M L, and Sitterley J Further stu lies of the geography of histopl min sensitivity in Kansas an l Missouri *J Kansas M Soc* 5 581 5 1 1951
5 Good R A an l Varc R I A clinical an l experimental study of agam na globul emia *J La cet* 13 215 291 19
6 Grayston J T an l Furcolow M I The occurrence f l t plasmosis in epi demics—ep lemiological studies *A n J Pul Health* 43 66 6 6 19 3
Gra ton J F an l Salv n S B Experimental histopla m sis in immunized and nonimmunized mice *Arch Path* 61 1 433 1956
8 Hill C A and Marcus S Nature of resi tance in mouse h topla mosi *I ct P ic* pt 81 87 19
9 Howell A Jr an l Kipkie C F A comparison of the su ceptil ility by intr cerebral in cul ati n cf six trai ns of mice with male ll a line l mi e A J *Tr p Med* 31 33 11 1 1
10 Lat zoff ky N A Fischer J B an l Han vas J J St l es on the anti en tru tu e of *Histopl capsul tum Ca ad J Mi l I* 3 9 9 19
11 Lan lsteiner K an l Chase M W Experiments on tran f r of cutaneou sen i t v ty to simple comp un ls *P oc S c E per P ol M d* 49 688 90 194
1 Looslı C. G Hi top la m sis Sc n e clini al epi le lo al an l l borat r a pect *Med Cl No th Am a* 39 1 1 19 19
13 Marcu S an l Hill C A Ext nt of res tan e in fected l m i nu at n aga t *Histoplas a capsulat m Fed I oc* 16 1 3 1 1 19
14 Marcus S an l Rambo F R Comparative a pect f the im u ation of m e a n t systemi m coses. *I act P o* f 9 19
1 Parsons R J and Zarafonetis C J D Hi t plast sis in n Report of se n cases and a review of seventy one cases *Ar l Int M I* 1 5 194
16 Raffel S Imm inity (properd n mn aglol uline a a lıation nd mmu logic paraly is) *A n J Ex Med* 38 414 19
17 R bi E, and Salvin S B Antigens from tl a t phase *H toplas a ap l m* I Morph logy of the cell a r eal l t the el tron microscope *F J C ll Resea cl* 10 591 101 19
18 R wles D A a d Huber M Crowtl f *H t plas a caps l t* n normal super fected a d immun zed m ce *J I n l* 15 3 19 6
19 Sal in S P E l toxin n path genic fungi *J Immu iol* 69 59 99 19
20 Salv S B Imm ization of mice ag n t *H t plasm cap l t m J I m nol* 0 67 0 19 3
21 Salvin S B Furth r stu lies on immun tion f mice a st *H t pl su lat m A J Hyg* 61 281 19
22 S lv n S B Resistan e to e nfe tion n experi ental histopla mos *J I mu ol* 4 14 221 19
23 Salvin S B Hypersen n t vi n m ce with e pe mental hi toplasmosis *J I mun l* 75 1 6 19
24 Salv n S B Acqu ed resistance in e pe mental histopla m is *T New Yo k Acad Sc* Ser II 18 46 468 1956
25 S lv n S B Occu rence of dela ed hypers n t v ty durin the development of Arthus type hype sensit t *J F per M d* 107 109 194 19 8
26 S lvin S B The influence of leu ocytes from en t zed mice on res tance to *H st plasma c f ulat A J Hyg* 68 255 41 1958

tions of variations in the natural resistance to the disease Fatal and nonfatal illnesses have occurred in all age groups About one fourth (25 7%) of the fatal illnesses reported from 1905 to 1951 occurred in infants under one year of age (12) With respect to age incidence was lowest in the group aged 10 to 19 years but was only slightly higher in the group aged 20 to 29 years The incidence was higher and somewhat variable in the older age groups The lower incidence of fatal illness in the teen age group may be due to one or more of the following factors (a) These individuals possibly because of their development of sexual maturity are naturally more resistant to infection than the other age groups (b) This group contains the greatest number of individuals who have most recently recovered from clinical or subclinical disease and therefore possess the highest degree of acquired resistance Since the greatest rate of conversion from negative to positive histoplasmin skin test frequently occurs in this age group or younger (4) recently acquired resistance may be the basis for its low incidence of fatality With regard to sex there *is no difference in incidence of histoplasmosis in the male* and female up to the age of 10 (12 15) Beyond age 10 males had a higher incidence of infection by a ratio of 4 to 1 This ratio parallels the situation in 8 to 14 week old mice where males were found to be more susceptible than females (30)

VACCINATION IN MAN

There is undoubtedly a need for a safe effective vaccine in certain areas of the country where the incidence is high and for certain high risk groups who for such reasons as age or occupation are more liable to infection Such a vaccine preferably should consist of a highly purified component derived from killed cells It should not be allergenic and therefore would not interfere with the histoplasmin skin reaction

REFERENCES

1 Chase M W The cellular transfer of cutaneous hypersensitivity to tuberculin *Proc Soc Exper Biol & Med* 59 131 13 1945

2 Day R Experimental ocular histoplasmosis *Am J Ophth* 32 1317 1330 1949

3 Farrell R L Cole C R Prior J A and Saslaw S Experimental histoplasmo

sis I Meth ls f r pr xluction f hist yl t t st In dom *I r c S c F fe Bi l & Me l 51* ,1 51 19 5

4 Furc l w M I an l Sitterley J Further stu lies f the geography f l t t j la min sen itivity in Kansas an l Mi souri *J Kansas M Soc* 5 91 9 13 1

5 Good R A an l Narc R I A clinical an l experimental stu ly f ranm a gl l ulmen ia *J Lancet 75* 91 ,1 19

6 Cray ton J T an l Furc low M I The occurrence f hi to lasm s i eq der nes—ep i len iological studies *A n J Pul H Ill 43* C C 19 5

7 Cras ton J I an l Salvin S B I xperin ental t istoj la i sis in immunize l n l n nimmunized mice *A ch I ath 61* 1 455 19

8 H ll C A an l Marcus S Nature of re i tan e t n euse hi to la n is *I t I roc* 11 81 8 19

9 Howell A, Jr an l Kipkie C F A c n parison f the su cept l il t Ly i itt cerel ral in oculati n f ix strains of mice ith m le ll a l ne l t *J Tr p Med 31* 33 11 19 1

10 Lal off ky N A Fischer J B an l Ham as J J Stu lies n tl e anti en t u ture of *Hist pla ria capsulatu Ca ral J Mi r* 3 9 0 1

11 Lan lsteiner K an l Chase M W Experiments on tran f rs of c itai eous sci si ti ity to sii ple comj un ls *I roc S c Exper Bi l M l 49* C88 00 191

1 Loosli C. C Hi toj l smosis Se me clinical ej le i l g al n l labor t r a pect *Me l Cl n N th A r a 39* 1 1 19

13 Marcus S an l H ll C A Extent of res tance m l cel l rat n agan t *Hi stoplas a caj lat m I el I ce 16* 1 3 1 1 19

14 Marcu S an l Raml F R Comparati e xpe t f l e n nu izati n f m c a ran t systemi i ycoses *Bact I c j* 9 19

1 Larsons R J a l Zarafonetis C J D Hi toj lasmosis in ı in Report of e i cases an l a r view of seventy-one cases *A h I l Me l* 5 1 3 191

16 Raffel S Immunity (properd n m aglol l nemia irra li tion an l t un m logic paraly ı) *A n Rev Me l 7* 58 411 19

1 R l F an l Sal ı S B Anti ens from tl e v a t pl ase *I H topla ia f t l n* I Morpl l ey f tl e c ll a r x l I tl el tron mic osc pe *I f Cell I es* rl 10 391 101 1

18 Rowley D A an l H iber M Gr tl *I H t f l caj lati n* n nor l uper nfecte l a l i nmi ized n ce *J I* 1 5 19

19 Salvin S P F til toxm m j th genic fungi *J I* 69 80 99 19

20 Salvin S B Imm ization f mice ag n t *H st plasma capsul tum J I ol* 0 0 19 5

1 Sal in S B Furtl er stu lies on i mmunization of mice again st *Hist pl af s l tu* l *J Hyg 61* 81 19

Salvin S B Resi tance to reinf cti n in e perimental histopla nosis *J I n* 14 1 19

3 Salvin S B Hypersen itivity in mice with experimental l istoplasm is *J I nu l 75* 1 6 19

4 Salvi S B Acquire l resista ce in experi iental histopla l osi *Tr New York Acad Sc* Ser II 18 16 168 19

5 Salvin S B Occurrence of del ye l hypersensiti ity h ng the development of Arthus type hyper ensitivity *J I xper Me l 107* 109 1 1 19 8

6 Sal in S B The influence l lei cocytes from se i it zed m ce on resi tance to *Hist plasn a c psul tum* 1 *J Hyg 68* 33 11 19 8

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REFERENCES

1 Chase M W The cellular transfer of cutaneous hypersensitivity to tuberculin *Proc Soc Exper Biol & Med* 59 134 13 1945

2 Day R Experimental *ocular histoplasmosis Am J Ophth* 32 1317 1330 1949

3 Farrell R L Cole C R Prior J A and Saslaw S Experimental histoplasmo

EPIDEMIOLOGY OF HISTOPLASMOSIS

MICHAEL L. FURCOLOW

The study of the localization and mode of spread of the fungus *Histoplasma capsulatum* has been one of the most fascinating problems confronting epidemiologists in modern times. Here one is presented with a disease which has infected 30 million people yet was practically unknown 13 years ago. Again, it is so strictly localized geographically that rates of infection may fall from 80% to 2% within a range of several hundred miles. This then cannot very well be a contagious disease nor can it be a disease with easily differentiated clinical symptomatology. All the evidence points to the fact that histoplasmosis is not a contagious disease in terms of being spread from person to person or from animals to humans. The failure of non-infected persons who contact patients to develop the disease or skin test sensitivity, lack of evidence of spread from humans to animals or vice versa, failure of spread of the disease from infected persons in areas of extremely low prevalence and the sharp localization of infection in epidemics to persons with specific exposure all support the hypothesis of non-contagion. Histoplasmosis then must be considered to be a disease of nature peculiarly localized to certain regions of the country and spread to humans from some reservoir in the soil or in nature. It is the purpose of this chapter to deal with the epidemiology of this interesting disease. This discussion will consider the subject under these headings:

1. Some Characteristics of Fungi Which are Important Epidemiologically
2. The Fungus and Its Environment
3. The Fungus and the Host

27 Salvin S B Unpublished results
28 Salvin S B and Furcolow M L Precipitins in human histoplasmosis *J Lab & Clin Med* 43 259 2/4 1954
29 Salvin S B and Ribi E Antigens from yeast phase of *Histoplasma capsulatum* II Immunologic properties of protoplasm vs cell walls *Proc Soc Exper Biol & Med* 90 287 294 1955
30 Saslaw S and Schaefer J Relation of sex and age to resistance of mice to experimental Histoplasma infections *Proc Soc Exper Biol & Med* 90 400-40? 1955
31 Schaefer J, and Saslaw S Some factors affecting resistance of mice to experimental histoplasmosis *Proc Soc Exper Biol & Med* 85 223 225 1954
3? Seltzer G Baron S and Toporek M Idiopathic hypogammaglobulinemia and agammaglobulinemia *New England J Med* 2?? 252 255 1955

Another characteristic of the fungi is their dependence for development upon satisfactory conditions of temperature and humidity. Weather conditions are used in both England and America to forecast the occurrence and spread of certain grain and vegetable blights. This will be further discussed under Environment.

Localized growth or geographic limitation of growth of fungi is well known. Some of the factors limiting growth are recognized while others are unknown. Thus it is well known that fungi of all kinds are prevalent in the tropics and rare in the colder climates. Here temperature and humidity seem to be limiting factors. In the case of certain tree and plant diseases the limiting factor is the presence or absence of certain insects which disperse the spores. Still other fungi are spread by the air and limited by the air currents. Very sharp geographic location has been shown by *Coccidioides immitis* and this appears to be due to certain temperature, humidity and rainfall characteristics which make up the Lower Sonoran Life Zone.

Histoplasma capsulatum has not been regarded as a particularly profuse spore bearer if one considers only the large tuberculated macrospores which are characteristic of the species. However, if one considers all spores produced by the organism (2) the production of spores is enormous. If one adds to this the infectiousness of mycelial fragments (3) it is readily seen that reproduction is well provided for. The fungus *H. capsulatum* is a slow grower and even on laboratory media of good quality is outgrown by most bacteria and fungi. Whether the organism produces an antibiotic to compensate for this slow growing has not been demonstrated. In common with the other fungi it appears that the spores of *H. capsulatum* are viable for long periods. Soil tests performed even a year after the collection of the soil frequently reveals viable organisms.

There is no question that *H. capsulatum* is disseminated in the air. The infection of persons in the laboratory in which the air-borne route is the most probable method of infection thoroughly demonstrates the dissemination of the organism by this route. Another characteristic of *H. capsulatum* is that the spores are viable for long periods in water as reported by Cooke (4) and Ritter (5).

The characteristic spores of *H. capsulatum* have long been

1 SOME CHARACTERISTICS OF FUNGI WHICH ARE IMPORTANT EPIDEMIOLOGICALLY

Profuse production of spores is one of the well recognized characteristics of fungi Ingold (1) in his book refers to estimates in the billions and indeed one species of bracket fungus was estimated to liberate 30 billion spores a day for an entire 6 month period A single colony of penicillin 2 5 cm in diameter may bear 400 million spores In addition to profuse production of spores the development of methods of dispersal of the spores by the fungi has been extremely varied and almost purposeful In most of the fungi the methods of dispersal of the spores consist either in the development of extremely light spores which easily float on the air the placing of the spore organs in such a location that any current of air will waft the spores about and disperse them and even the development of special dispersal organs which more or less snap the spores out into the air thus giving them an airborne start Indeed so common is this forcible ejection of the spores into the air among the fungi that a special name has been developed called *delissing* to describe this process Another characteristic of the spores is the fact that they are not harmed by drying and they are viable for extremely long periods of time It is well known that the spores of certain plant and fruit diseases may lie dormant but viable for several years in the ground or on leaves or plants until such time as growth conditions are favorable Spores are extremely resistant organisms especially protected from the harmful effects of nature by thick coats thus they are particularly resistant to heat drying and other physical conditions which readily destroy the organism in its more sensitive stages or other organisms

Still another property of the fungi is the tendency to produce antibiotics as inhibitors of other fungus or bacterial growth and some of the growth substances produced are not only inhibitory but definitely lethal to other organisms Particularly well known antibiotics are penicillin and streptomycin The production of these antibiotics would appear to be compensatory mechanism to protect the organism and give it a better chance to survive It should be stressed however that the production of deterrent substances by one organism for another is widespread in nature

abundant spores are found in the range of 1 to 5 microns in size (Fig 1) It should also be mentioned that these experiments dis regarded the mycelium which was later shown by Larsh (3) to be potentially infectious It is quite evident therefore that *H capsu latum* does produce infectious elements of the size quite capable of entering into the alveoli of the lungs and being retained there Recent work by Larsh and his associates (3) indicates that all of the elements of *H capsulatum* that is macrospores microspores and mycelial elements are viable and potentially infectious Thus Table I produced from Larsh s publication shows that the 5 ele ments are all infectious for mice or chick embryos or both When one considers the low viability of the spores as found by these experi

TABLE I

PERCENTAGE OF WHITE MICE AND CHICK EMBRYOS INFECTED BY SINGLE PARTICLE INOCULUM OF 3 MONTHS OLD CULTURE OF THE ELLIS ISOLATE OF HISTOPLASMA CAPSULATUM

Units Inoculated	*White Mouse*		*Chick Embryo*	
	Infective Ratio	*% Infected*	*Infective Ratio*	*% Infected*
Non-germinated tuberculated macroconidia	4/48	8.2	0/40	.0
Non germinated microconidia	1/47	2.1	3/4	7.1
Germinated tuberculated macroconidia	3/19	6.2	/51	13.7
Germinated microconidia	1/19	5.3	3/51	5.9
Non branching mycelial fragments	0/20	0	4/28	14.3
Total	9/132	5.0	17/212	8.0

(Courtesy Larsh *et al* *Proc Soc Exper Biol & Med* 93 19 8)

$$x/n = \frac{\text{No of Animals Infected}}{\text{No of Animals Inoculated}}$$

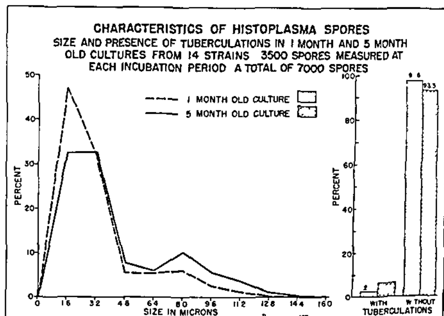


Fig 1

known to be large and tuberculated indeed the original descrip tion of DeMonbreun clearly illustrates the size variation of the macrospores from 5 to 15 microns (6) However as has also been pointed out by DeMonbreun (6) the tuberculated macrospores are not the only spores there are also both round and pear shape macrospores which are non tuberculated In addition to the micro spores which are sometimes tuberculated and more often non tuberculated there is the mycelium itself which fragments and disseminates when dry

When one considers the characteristic macroconidia which are so large one wonders how the spores might enter the respiratory tract It has been shown by Brown *et al* (7) that particles of the size of 5 microns or more are usually filtered out of the air before penetrating beyond the larger bronchi This would suggest that the macrospores of *H capsulatum* probably are not the infectious agents in man or animals However in the previously recorded experiments of our laboratory by Cozad *et al* (8) and Helmbright and Larsh (2) *it is amply evident that the macrospores make up only* a small portion of the spores of H capsulatum and that the most

Most of the systemic fungi have a wide host range infecting both men and animals No infections of plants by *Histoplasma* have been demonstrated but in addition to man a wide variety of animals have been infected either naturally or artificially This subject is covered in the chapter on animal infection Natural infection seems to be most common among dogs and cats as reported by Emmons (11) and among cattle horses and sheep (12) Fig 3 compares infection rates in cattle horses and sheep with children in the same county It is seen that the rates are quite similar on an age specific basis Also as seen in Fig 4 these rates vary with the rates in the residents in the same area Thus the rates in Tennessee Iowa Kansas and Missouri among cattle and among humans in the same county tend to resemble each other very markedly These data will be commented on later in regard to the theories of the spread of infection

As discussed in the chapter on Morphology and Physiology *H capsulatum* grows in two phases depending upon the temperature and environment The mycelial phase is extremely resistant

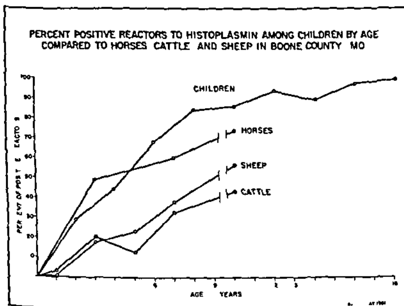


Fig 3

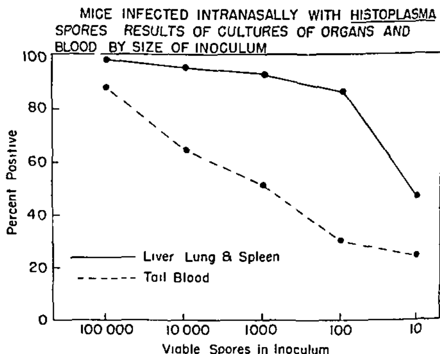


Fig 2

ments one wonders how persons ever become infected However it must be remembered that the viability in human beings may be quite different from the viability on laboratory media and experiments in this laboratory by Grayston and Altman (9) suggest that most of the spores are infectious if placed in the lungs under proper environment Fig 2 reproduced from unpublished work of Grayston and Altman shows that the spores are readily infectious to mice even in relatively small doses Also Ajello (10) has shown that under the conditions of his experiments 100% of the tuberculated macroconidia were infectious for mice when implanted intraperitoneally accompanied by the agar plug upon which the organisms were growing These seemingly contradictory laboratory findings can probably best be explained by saying that we are not yet fully acquainted with the infectiousness of the spores and that it probably varies under different conditions being fairly low on artificial media but from all appearances much higher in human or animal hosts

histoplasmosis which occurred in a silo in Northern Indiana. In 1952 Zeidberg (15) and his co-workers first called attention to the frequency of isolation in chicken yards and about chicken houses and the infrequency of isolation from any other areas about the farm. Table II reproduced from their publication (16) shows the results of 319 samples collected in Tennessee. They also called attention to the fact that sheltered samples were more commonly positive than open samples. Emmons (17) and Ajello (18) have also reported extensive soil sampling and isolations. In Table III is shown the percentage of isolations from 1024 soils studied at this Station by the type of natural environment from which soil collections were made. It is evident that the frequency of isolation is not only related to the type of environment but to the degree of histoplasmin sensitivity in the general area. The highest frequency of isolation (27%) was obtained from chicken coops in the high area of histoplasmin sensitivity. However, as shown in Table IV, the

TABLE II

RESULTS OF EXAMINATIONS OF SOIL SAMPLES COLLECTED FROM 8[illegible] PREMISES BY SOURCE OF SAMPLE, WILLIAMSON COUNTY, TENNESSEE, JULY 19[illegible]0–OCTOBER 19[illegible]1

Source of Sample	*Number of Samples*	*Histoplasma capsulatum isolated*	
		Number	*Per Cent*
Total	319	11	3 2
Under house	58	4	6 9
Near house	103	0	
Inside chicken house	47	5	10 [illegible]
Chicken yard, coop, etc.	18	[illegible]	11 1
Barn yard	30	0	
Inside barn	9	0	
Bank of water course	0	0	
In open	[illegible]8	0	
Other	6	0	—

(Courtesy L. D. Zeidberg, M.D., Pub. Health Monograph No. 39, 1956)

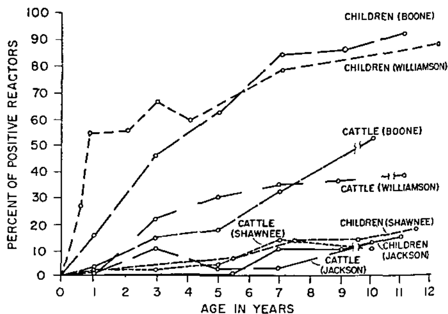


Fig 4

to drying and heat and other physically unfavorable environments while the yeast phase is extremely susceptible to changes in environ ment readily being killed by drying freezing and other physical means The moist capsule of the yeast combined with its small size of 2 to 4 microns probably account for its low resistance to unfavor able conditions

2 THE FUNGUS AND ITS ENVIRONMENT

Knowledge of the growth of *H capsulatum* in nature dates from the first isolation by Emmons (13) in 1949 when he isolated the organisms from the soil about a rodent burrow under a chicken coop In 1951 Grayston Loosli and Alexander (14) reported the first isolation of the organism in connection with an epidemic of

histoplasmosis which occurred in a silo in Northern Indiana. In 1952 Zeidberg (15) and his co-workers first called attention to the frequency of isolation in chicken yards and about chicken houses and the infrequency of isolation from any other areas about the farm. Table II reproduced from their publication (16) shows the results of 319 samples collected in Tennessee. They also called attention to the fact that sheltered samples were more commonly positive than open samples. Emmons (17) and Ajello (18) have also reported extensive soil sampling and isolations. In Table III is shown the percentage of isolations from 1021 soils studied at this Station by the type of natural environment from which soil collections were made. It is evident that the frequency of isolation is not only related to the type of environment but to the degree of histoplasmin sensitivity in the general area. The highest frequency of isolation (27%) was obtained from chicken coops in the high area of histoplasmin sensitivity. However, as shown in Table IV, the

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Source of Sample	Number of Samples	*Histoplasma capsulatum* isolated	
		Number	Per Cent
Total	31[illegible]	11	[illegible]
Under house	8	4	[illegible]
Near house	103	0	
Inside chicken house	47		10.[illegible]
Chicken yard, coop, etc.	18	2	11.1
Barn yard	0	0	
Inside barn	9	0	
Bank of water course	[illegible]	0	
In open	23	0	
Other	[illegible]	0	

(Courtesy L. D. Zeidberg, M.D., Pub. Health Monogr. [illegible])

TABLE III

PERCENTAGE OF SOIL ISOLATIONS BY TYPE OF NATURAL ENVIRONMENT FROM WHICH COLLECTIONS WERE MADE (1024 SAMPLES)

Histoplasmin Sensitivity	*Collection Site*				
	Covered Moist Caves Silos Storm Cellars	*Open Moist Woods Stumps River Banks*	*Around Houses*	*Around Barns Except Chicken Coops*	*Around Chicken Coops*
0 10	0	0	0	3	0*
10 35	40	6	0	2	0
15 5	6		[illegible]	10	
0 90	3	[illegible]	1	1	27
All	3	[illegible]	1	4	16

*20 soils or less tested

TABLE IV

PERCENTAGE OF SOIL ISOLATIONS BY INFECTIOUS HISTORY OF THE SITE OF COLLECTION (10[illegible] SAMPLES)

Collection Site	*Histoplasmin Sensitivity*				
	0-10	*10-35*	*45-55*	*70-90*	*All*
Random	0	4		6	3
Sites of epidemic	7	8	7	[illegible]	12
Associated with cases		0	6	15	9
Total all soil collected anywhere	1	3	7	7	5

frequency of isolation differs not only with the degree of histoplasmin sensitivity but also with the infectious history of the environment from which the soil collections were made The frequency of isolations among the random collections is only about 3% whereas among soils collected from sites of epidemics or their immediate neighborhood it is 4 times as high or 12% These two tables illustrate 3 points first what appears to be a relationship of geography with a frequency of isolation second the fact that isolations are more frequent around the sites where epidemics or cases have occurred and third that the natural environment plays a considerable part in the frequency of isolation with a definite relationship being shown between chickens and the frequency of chicken habitation to the frequency of isolations In our experience the open and closed areas have made very little difference in the frequency of isolation although few if any isolations are made from areas directly exposed to sunlight

As mentioned before one of the characteristics of fungus infections is their geographic localization Indeed this is one of the most distinguishing characteristics of histoplasmosis This variation

in geographic prevalence is best brought out by skin test sensitivity surveys (see chapter on Skin Test Sensitivity (19) It can be said however that there are marked geographic variations in the frequency of reactions and that in general the most highly endemic area is fairly sharply localized to the Central river valleys of the United States The prevalence of high rates of infection along the river valleys of the Ohio Missouri and Mississippi rivers has long been cause for comment indeed this geographic prevalence along the river valleys may be carried to many of the river valleys of the world Infection has been demonstrated in the Amazon La Plata and Orinoco river valleys in South America along the Congo river and Niger river in Africa and along the Irrawaddy river in Burma Even in the United States when one looks carefully at the sensitivity maps one finds that the prevalence of sensitivity outside of the very high areas of sensitivity may still be traced to river valleys such as the Lake Champlain and St Lawrence river valleys in New York State the Susquehanna river valley in Pennsylvania the Potomac river valley in Maryland and Virginia and the Rio Grande and Pecos river valleys in Texas Indeed the extension of sensitivity across the Appalachian Mountains may even be found to occur in the Broad river valley in the North Carolina and Tennessee area In view of this widespread correlation of *Histoplasma* with the river valley areas one might suspect that the infection would occur along the Mekong in Indo China and Cambodia the Ping river in Thailand the Indus and Ganges river basins in India and the Yangtze and Yellow river basins in China Most infections with *H capsulatum* have been reported from the area of 45 degrees north to 30 degrees south of the equator

There are several theories proposed to explain this geographical limitation one of them being that the sensitivity coincides with a certain life zone Maddy (20) has shown a distinct overlapping of the lower Sonoran Life Zone with the distribution of *Coccidioides* infection With *Histoplasma* no such life zone phenomenon could be demonstrated since the area of high infection overlaps several life zones

Another theory is that soil groups are the limiting factors in the geographic prevalence of the disease The leading proponent of

GEOGRAPHIC VARIATIONS IN HISTOPLASMIN SENSITIVITY



SOURCE ADAPTED FROM SOILS AND MEN YEARBOOK OF AGRICULTURE 1938 BY PERMISSION

FIG 2 Distribution of red yellow podzolic soil in the United States

Fig 5

this theory is Zeidberg (21) who has postulated that the distribution of red yellow podzolic soil conforms fairly well with the distribution of *H capsulatum* The distribution of red yellow podzolic soil in the United States is shown in Fig 5 and in the world in Fig 6 (See chapter on Skin Test Sensitivity for world distribution of histoplasmin sensitivity) There is indeed considerable correlation between the skin sensitivity and this particular soil group but there are many areas in which this soil occurs and histoplasmosis does not occur and vice versa Positive soils have shown higher acidity organic content and moisture holding capacity than negative soils (22) but these findings again only lead us back to contamination of these soils with chicken droppings

Emmons (23) originally proposed a theory similar to that which he had proposed for *Coccidioides* namely that the organism was endemic among certain animals whose geographic distribution limited the spread of infection Emmons has isolated the organism from numerous wild and domestic animals both in and outside of

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disease by birds since it appears the infection tapers off both at the extreme lower end of the Mississippi Valley in Louisiana and at the upper end of the Missouri Mississippi and Ohio valleys Also tests of wild birds for infection by culture or serology have been uniformly negative (24 25)

A theory of localization to river valleys by the presence of water (26) has been advanced Identification of the organism from water by Gordon (27) and determinations of its viability in water by both Ritter (5) and Cooke (1) as mentioned previously require water to be considered However the decrease in infection at both the upper and lower ends of the river valleys and the lack of direct connection to water are opposed to this In Kansas City Chicago and Cleveland for example rates of infection in the cities located on the waterfront are lower than in their surrounding rural areas

Another theory of interest regarding the localization has to do with the possible growth of the organism as a parasite on certain plants whose distribution might be limited to the high areas of sensitivity Experiments by Furcolow and Horr (28) on certain specimens of plants brought into the laboratory and inoculated artificially with *H capsulatum* led to the conclusion that the temperature and humidity conditions in the local area were more important to the growth of *Histoplasma* than was the substrata upon which it was growing Thus when a specimen of plant was put in a petri dish and inoculated and the petri dish was kept too wet the fungus would not grow On the other hand if it was kept too dry the fungus would not grow However if damp filter paper was kept in the petri dish and the petri dish was kept at room temperature the growth on the specimen was quite satisfactory as illustrated in Fig 7

The control of the growth of fungi by humidity and temperature has been commented upon The preference of the fungi for moist humid conditions and warmer temperatures is well known and illustrated by the extremely frequent occurrence of fungi under natural conditions more particularly in the tropics The growth of *Histoplasma* does seem to be affected by certain temperature and humidity conditions In Fig 8 is shown the extremely limited conditions under which *H capsulatum* will grow in soil in the

GEOGRAPHIC VARIATIONS IN HISTOPLASMIN SENSITIVITY

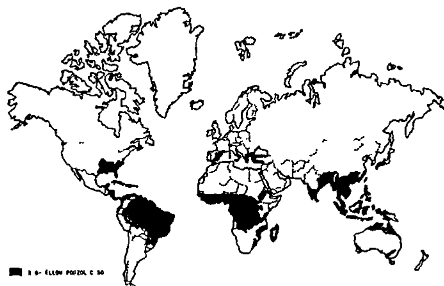


Fig 6

the endemic area (see chapter on Natural Reservoirs) Whether animals serve as the reservoir must await further study but the similarity of skin test rates in animals and humans in the same area as mentioned earlier does not support this theory

Another interesting theory relates to the possibility of wild birds being important in the localization of infection to the high sensitivity area However the very fact that many birds migrate has made it difficult to accept the importance of birds to so limited an infection It is important to remark however that more than half of the epidemics have been associated with sites in which excreta of either chickens pigeons or bats was involved Indeed in 88 per cent of the epidemics either rural environment or bird excreta was definitely involved Another interesting thought in regard to bird migration is that the main migratory pathways of the birds in the Central United States are along the Mississippi valley where the prevalence is high Many of these birds migrate to the Amazon Valley of Brazil where infection is also known to be endemic However the fact that the infection is not more widely spread up and down the Mississippi valley is against the spread of the

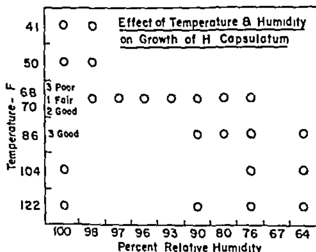


Fig 8

—Percentage of Children under 15 Years of Age Positi e to Histoplasmin accord ing to Damp ess of Environment by Age G oup Williamson County Tennes ee 1946

Fig 9

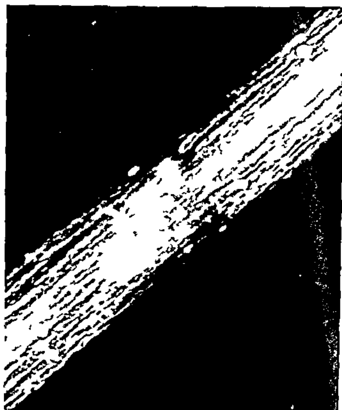


Fig 7

laboratory It is seen that no growth took place in less than 100% relative humidity and that growth was also strictly controlled by temperature extending only in the range from about 68 to 86° Zeidberg *et al* (29) had shown earlier a definite relationship between the dampness of the environment and the frequency of positive histoplasmin skin test reactors (Fig 9) Consideration of these factors in reviewing the evidence previously reported of the frequency of finding the organism in chicken coops and environments *where chickens were living suggested either that chickens* were an important source of the organism or that they furnished an environment which was suitable for the growth of the organism

Extensive investigations of the possible role of chickens in histoplasmosis have all been uniformly negative Emmons (17) Zeid

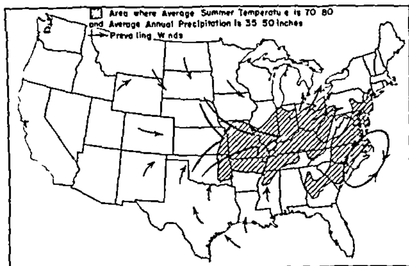


Fig 11

to be some proof of the prevalence of satisfactory environmental conditions in chicken coops

It is interesting that studies of weather bureau records reveal some interesting information regarding the temperature and humidity in the United States and the distribution of histoplasmin sensitivity In Fig 10 is shown the frequency of satisfactory days fulfilling certain temperature and humidity requirements and based on weather bureau records for the year 1950 (31) This shows that in the region of South Missouri and Arkansas the frequency of satisfactory days is highest and that this frequency decreases as one moves away from this area Another such calculation based on the 50 year records of the Weather Bureau is shown in Fig 11 which indicates the areas in the United States which certain conditions of temperature and humidity delimit It is noted that west of the Appalachian mountains the area in general approaches the highest histoplasmin sensitivity area East of the Appalachian mountains there appears to be an area of satisfactory conditions in most of which *H capsulatum* apparently does not frequently grow However in the north part in the area around Washington D C and up into Maryland Pennsylvania and Delaware recent evidence

berg *et al* (15) Menges and Haberman (30) Schwarz *et al* (25) and others have participated in these studies Not only are skin test positive chickens rare in known infected environments but autopsy of such chickens fails to reveal the organism Similarly most chickens artificially infected by a variety of routes not only do not become ill but are negative to culture on autopsy Finally cultures of the feces of these artificially infected chickens have been uniformly negative It does not seem therefore that chickens are either infected or carriers of the fungus It is still possible and indeed likely that the chickens are only indirectly concerned in that chicken coops furnish a satisfactory environment for the organism to grow This is especially so when one remembers that the moist humid conditions ordinarily prevalent in chicken coops pigeon lofts and other such bird harborages which one could easily imagine could be ideal for the growth of the fungus Studies by Becker (31) have shown adequate temperature and humidity conditions for growth of *Histoplasma* occurred in 2 chicken coops in Iowa for 10 to 20 days a month during 7 months beginning May 1955 Thus there appears

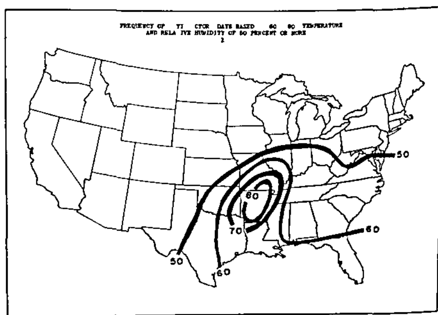


Fig 10

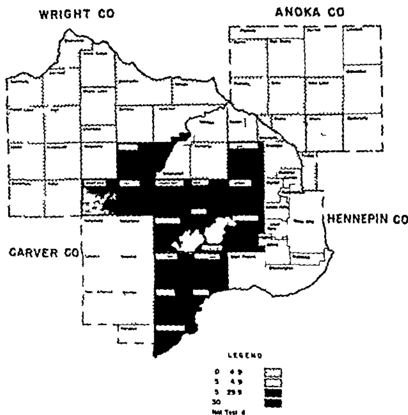


Fig 12

mal to each other Similar variations in degrees of prevalence in a relatively small area are found in Jackson County Iowa (Fig 13) Here it is seen that the rates in the Eastern part of the county along the Mississippi river are less than half of the rates in the westward more interior parts of this country (12) The presence of epidemics in various parts of the country remote from the areas of high sensi

does indicate that histoplasmosis exists with a fair degree of frequency (32 33 34) Also in Georgia (35 36) and in North Carolina (37) areas of prevalence of infection have recently been demonstrated It is thus evident that even with the crude figures now available some correlation between weather conditions and the prevalence of the fungus in the environment can be demonstrated In this figure the direction of the prevailing winds is indicated and might explain the infrequency of dispersion of the fungus spores across the Appalachian mountains The finding of small foci of infection at infrequent intervals in the areas of low sensitivity to the north (Minnesota and Iowa) and to the north east of the high area (Lake Champlain area) (38) also suggests less frequent spore dispersion over these areas which agrees with frequency of the prevailing winds from the high area Other explanations are possible for these infrequent foci such as less favorable general environmental conditions in these areas or indeed many others

The presence of the prevailing winds actually brings to mind another question and that is how is the disease spread about in nature? There are three theories in this regard the first being that mentioned above namely spread by the prevailing winds the second and earlier theory of Beadenkopf and Loosli (39) which proposed that the spores are carried by floods along the river valleys and then after recession of the flood waters the organism is disseminated by the wind from the dried up floodland Still another theory is that of Manos (40) who postulated that tornadoes were important in dispersing the organism about in nature This will be discussed when the infection of persons is discussed

The question whether *H capsulatum* is limited in its natural habitat to the areas where histoplasmin sensitivity in humans is high or whether it is dispersed throughout wider areas is indeed fascinating It is quite evident from studies in widely scattered parts of the country that the organism is present in certain areas even of general low sensitivity (35) but that its prevalence is distinctly of a localized nature and not generalized as it is in the highly endemic areas Fig 12 shows the distribution of sensitivity in townships about the city of Minneapolis (41) It is quite evident that the sensitivity varies from less than 5% to more than 30% within areas very proxi

THE TULSA TORNADO

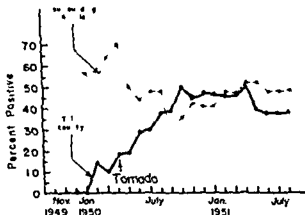


Fig 15

tivity also leads one to suspect that the prevalence of the disease is not localized to the high areas (Fig 14) (13)

The isolation by Emmons of *Histoplasma* (35) from skunks in Georgia in areas where the sensitivity among humans is very low also supports the evidence that the organism may be widely distributed in nature perhaps with lower frequency Aronson s (36) work in Georgia also supports this

The problem of the limitation or dissemination of the fungus throughout the environment may also be approached by consideration of where people get infected The basic question here is whether people become infected by the fungus blowing about generally through the air or whether they become infected by visits to or contacts with local sources where the fungus is growing This first method of infection is commonly regarded to be that which occurs in coccidioidomycosis where wind blown dust contaminated with spores is regarded as the method of infection and it is considered that this infection can be acquired at any place throughout the broad expanse of the endemic area With histoplasmosis this theory is by no means proved The main exponent of generalized infection has been Manos who has presented evidence that tornadoes can infect people This problem was first studied by follow

HISTOPLASMIN SENSITIVITY IN JACKSON COUNTY IOWA

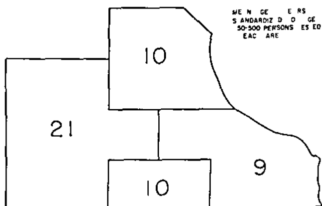


Fig 13

Location of Epidemics of Histoplasmosis in Relation to Prevalence of Histoplasmin Sensitivity

Less than 10
10 30
30 80
Ove 80

Fig 14

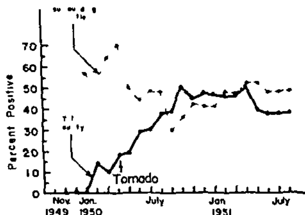


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ing the sensitivity to histoplasmin of navy recruits from an area where a tornado occurred prior to and after the tornado In Fig 15 is shown histoplasmin sensitivity among Navy recruits from the area of Tulsa Oklahoma from November 1949 through July 1951 The groups compared are from Tulsa county and from the surrounding counties A tornado occurred in Tulsa county in April 1950 It is seen that the sensitivity of the men from the surrounding counties is considerably higher than that in Tulsa which is not unexpected since rural sensitivity has generally been shown to be higher than urban (40) In Tulsa county the sensitivity in general is rather low but starts to rise about February 1950 2 months before the tornado This rise continues uninterrupted until about 6 months after the tornado after which the sensitivity for the Tulsa recruits is the same as that for the surrounding counties

Following these studies tests were performed in three counties in Mississippi following a tornado in February 1955 Histoplasmin tests were performed in various schools in the three counties in February immediately following the tornado and 9 months later in November of 1955 In Fig 16 is shown the resulting comparisons of the increase in percentage of reactors in various schools in these counties in the 9 months following the tornado It will be noted that there was a marked increase in all of the schools in Tunica county whereas in De Sota and Tate counties there was little or no increase in sensitivity It does seem peculiar that if the increase in sensitivity in Tunica county was associated with the

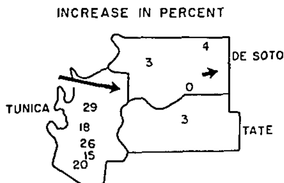


Fig 16

tornado there was not a similar increase in the other counties since as is evident from the arrows the tornado pursued a course through both areas It is quite apparent that this is not yet acceptable as definite evidence of infection by tornadoes

The second theory postulates that people are infected from point sources where they come in contact with some focus of growth of the fungus such as a chicken coop or other suitable place Some of the material already presented indicated that the organism is not widely distributed in nature but localized to point sources particularly point sources associated with chickens A still more important bit of evidence in this regard is the occurrence of the epidemics As was shown in Fig 11 more than 10 epidemics have now been reported The epidemics do not necessarily occur in high histoplasmin areas but almost all have shown definitely to be associated with the presence of *H capsulatum* at a point source The evidence indicates that these persons are infected by coming in contact with a point source where the organism is growing The illness in the epidemics has often been severe although investigation often brings cases of mild infection to light It has been shown quite clearly (14) that the number of lung lesions and degree of illness can be correlated with the degree of exposure at the point source and presumably with the number of organisms inhaled This suggests that perhaps many or most of the casual infections are actually epidemics in miniature with the persons being exposed at the point source to varying degrees of contamination and developing varying degrees of pulmonary infiltration and illness Study of certain epidemics in particular (15) shows that the x ray pictures in a given epidemic may vary from extensive widespread infiltrations to occasional nodular densities or enlargement of the hilar lymph nodes It is quite possible therefore that infection among persons even in the highly endemic area occurs only among persons who are exposed to local spots where the fungus is growing such as chicken coops pigeon lofts etc

With this thought in mind it might be well to view some of the evidence collected by questionnaire from the Kansas City studies (16) dealing with the source of infection of more than 6 000 children residing in Kansas City and attending the kindergarten or first

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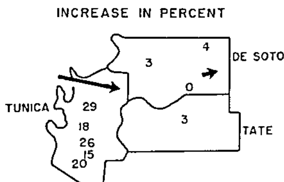
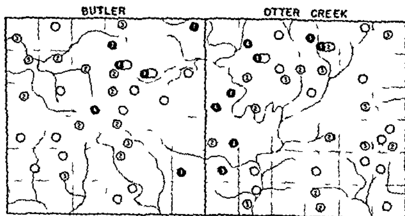


Fig 16

whereas among those who were most distant from rural areas only 3.4% showed such positive reactions. This again suggests that the proximity to rural environment increases the chances of exposure to the fungus. Another point of interest in regard to this population of city dwellers is that the rates of reaction among those denying visits to farms or living on farms was much higher in the older sections of the city than in the new sections. Whether this relates to the age of the dwelling, the possible deterioration of the wood caused by fungi, or the proximity of this section of the city to the railroad yards and other known sources of bird harborages remains in question. That this might be the case was supported by figures obtained from another study on an army post (37) in which the acquisition of sensitivity among children previously negative to histoplasmin was studied. Among these negative children who lived in homes where pigeons lived on the roof of the house 5% showed infection compared to 2.2% of infections among children who lived in houses where no such pigeon harborage was noted. Additional evidence of the relationship of increase in sensitivity with exposure to pigeons is shown in the epidemics (43). These



ONE C LD N HOUSEHOLD STOPLASM N POS T VE
TWO C LDREN IN HO SEHOLD BOT S OPLASM N NEGATIVE
THREE CHILDREN IN HOUSEHO D ONE IS OPL SM N POSITIVE

Detailed m p of th two norther mo t town hips of th we tern a ea of figure 2 sh wing hi t plasm t n am g childr n i f rm ho seh lds. Each ci cl rep esents a i d id l f rm and hows th mbe f h ld en te ted

Fig 18

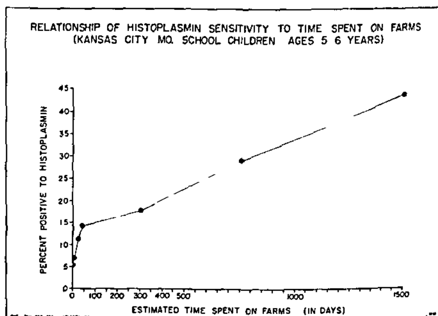


Fig 17

grade It is quite obvious that among such children many will have either lived or visited on farms and actually in Fig 17 the calculation has been made of the estimated number of days spent on the farm in relation to histoplasmin sensitivity among these city school children Among this group infection could not be traced to any specific animal contact on the farm This perhaps is not unexpected since one would be surprised if children who had visited on farms would remember any specific contact with chickens or other animals However it is quite likely that such contact occurred even though denied

It was noted that almost 2700 children denied ever living or visiting on a farm and 111 or 5 1% of these were found to be positive to histoplasmin Studies were instituted as to where these children might have contracted their infection It was extremely interesting to compare the proximity to rural areas of the schools which these children attended When those 10 schools most proximal to rural areas were compared with the 10 schools most distant from rural areas it was found that among the schools proximal to rural areas 7% of the children showed histoplasmin reactions

the skin test rates in the same city (58) reveals that apparently lesions were found with equal frequency with skin test sensitivity. In other words almost every skin test positive person had demonstrable pulmonary lesion. This is indeed strong evidence of respiratory infection.

Similarly, although mucocutaneous lesions are not at all uncommon in histoplasmosis, it is usually found that such lesions are secondary, accompanying blood borne dissemination of the organisms and not primary. That most patients are infected by the air borne route is supported by the epidemics where the air borne inhalation of spores was related to activities at the point source. The duration and degree of exposure was correlated to the severity of illness and the frequency of lung lesions. Similarly, among laboratory personnel who worked with the fungus it has been found that the infections are very common (54) although usually of a more mild degree. It should also be mentioned that the organism has been isolated from the air in areas where epidemic cases had occurred (12). Animal experimentation clearly indicates that the most infectious route for *Histoplasma capsulatum* is via the respiratory tract. Mice infected with as few as 10 spores regularly showed lesions and disease in experiments performed by Grayston and others in our laboratory (9).

It is interesting to make comparisons of the pathogenicity of the various fungus infections and tuberculosis. It is important to realize that the rate of calcification found in the lung by x ray with Histoplasma infection is higher than that of any other known infection. This is demonstrated by comparing the rate of the finding of calcified lesions in persons positive to histoplasmin with the rates in persons positive to coccidioidin or tuberculin. About one third of the histoplasmin reactors will have calcification (56, 57) compared to 14% of the coccidioidin reactors (58) and 10% of the tuberculin reactors (56). It is also interesting to note that in contrast to *Coccidioides* there is more of a problem of recurrent infection in the older age groups although still less than with tuberculosis.

Another point of interest might be whether most infections are single or multiple. Actually this matter has not been intensively studied but judging from x rays of many surveys in schools and

studies suggest that exposure to point sources most often in rural environments is the most important source of infection and that little if any infection is produced by widespread blowing of the spores

These studies are further supported by the demonstration of the localized distribution of infection in various counties in different parts of the country where sensitivity is low An example of this type of relationship is shown in Fig 18 (also see Fig 13) based on skin testing carried out in the schools of Jackson County Iowa where the spotty distribution of positives is clearly evident It is seen that sometimes a family with three or four positive reactors may reside within a mile of a family with three or four negative reactors This is supported by other studies (Sachs *et al* 48 Zeidberg *et al*, 15) The evidence suggests that in areas of low sensitivity at least the distribution of the organism is extremely localized and appears to be associated with point sources rather than generalized distribution This suggests that people get infected by coming in contact with these localized areas of fungus proliferation

3 THE FUNGUS AND THE HOST

The route of infection in histoplasmosis could be either oral or respiratory or both The oral route has been suggested by the finding of the organisms in water (27) the occasional occurrence of gastro intestinal cases especially in children (19) and the finding of splenic calcification in many cases Indeed Straub and Schwarz (50) showed that 14% of spleens showed calcification in Cincinnati compared to 3% in New York and 2% in Rotterdam However Grayston in our laboratory was unable to infect mice by gavage whereas intranasal infection was common (9) Cole has had the same experience with dogs (51) The second point against oral infection is the frequency of lung lesions and their variety which suggests that the lesions are not blood borne but that the portal of entry is directly into the lungs Indeed Straub and Schwarz (52) have demonstrated by careful pathologic studies that calcified pulmonary lesions of a size that could be detected at autopsy were found in three fourths of the lungs examined in Cincinnati Comparing these rates with

In regard to pathogenicity of the organism it appears from the evidence presented by Schwarz that the organism in general after invading the lungs tends to become disseminated. This is supported by the frequent finding of calcifications in the spleen among persons otherwise healthy who have positive histoplasmin skin tests. However the finding of lung calcifications or evidence of infection accompanying the splenic calcification points toward a pulmonary source of entry rather than gastrointestinal.

When one considers the epidemiologic implications of the frequency of disease by sex, age, race and year of recognition the remarkable strides of recent years become apparent. Fig. 19 shows the number of cases reported through the years beginning in 1905. The data on cases prior to 1950 was adapted from Loosli (60). However since 1950 only cases were accepted which had positive results by culture or pathologic examination.

Prior to 1935, as is well known, only a few cases were reported. The first 3 cases of Darling (1905 and 1906) were followed by a gap of about 20 years until the first case in America was reported in Minnesota by Riley and Watson (61). In the following 10 years only 10 cases were reported. All the cases reported until 1935 were fatal.

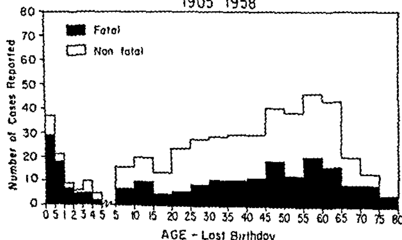


Fig 20

among young persons it is seen that many infections are single with the resultant either a nodule in the lung or Ghon complex with a nodule in the lung and enlargement of the hilar lymph nodes On the other hand more than one primary lesion is not at all uncommon and indeed it is our impression that multiple lesions are much more common than with either tuberculosis or coccidioidomycosis Reinfection unquestionably seems to be the explanation for the chronic cavitary lesions with apical location found among older persons with increasing frequency Approximately 100 cases have now been discovered in one sanatorium in Missouri (59) Cases are being reported from sanatoria throughout the country so that it is unquestionably true that cases of this type are common and have been previously misdiagnosed as tuberculosis

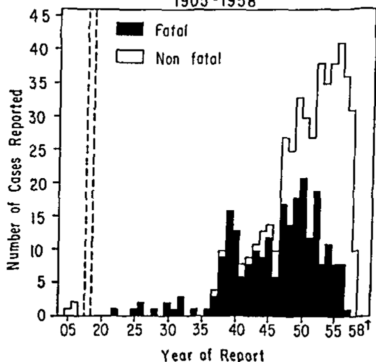


Fig 19

there is a different causation for these two peaks since the younger peak is probably due to fatalities associated with first infection while the latter peak is probably due to endogenous reinfection

In Table V are shown the reported cases by race and sex as well as by place of residence of the patient It is seen that 121 of 491 cases were females or 25% and only 13 were non white Of the 491 cases only 50 or 10% were from foreign countries It is also seen that 80% of the United States cases and 86% of the foreign cases were over 10 years of age It is unquestionable that there are other areas of the world where histoplasmosis is extremely prevalent It appears also that the low percentage of reports from the foreign literature does not represent absence of cases in many areas but failure of recognition and reporting It is easy to predict not only increasing recognition of the disease in this country but in many foreign countries as knowledge of this most elusive fungus infection becomes more widespread

REFERENCES

1 Ingold C T *Dispersal in Fungi* Clarendon Press Oxford England 1953

2 Helmbright A L and Larsh H W The size of the spores of *Histoplasma capsulatum* *Proc Soc Exper Biol & Med 81* 0 5 1 19 2

3 Larsh H W Scholes V E Hinton A and Silberg S The minimal infectious inoculum of *Histoplasma capsulatum* for the mouse and chick embryo *Proc Soc Exper Biol & Med 98* 5 0 573 19 8

4 Cooke W B and Kabler P W The survival of *Histoplasma capsulatum* in water *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 19 6 pp 261 264

Ritter C Studies of the viability of *Histoplasma capsulatum* in tap water *Am J Pub Health 44* 1299 1301 Oct 19 4

6 De Monbreun W A The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum* *Am J Trop Med 15* 93 1 5 1934

Brown J H Cook K M Ney F G and Hatch T Influence of particle size upon the retention of particle matter in the human lung *Am J Pub Health 40* 450-4 9 1950

8 Cozad G C and Furcolow M L Laboratory studies of *Histoplasma capsulatum* II Size of the Spores *J Infect Dis 9* 77 84 19 3

9 Grayston J T Altman P L and Cozad G C Experimental histoplasmosis in mice A preliminary report *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 19 6 pp 99 105

10 Ajello L and Runyon L C Infection of mice with single spores of *Histoplasma capsulatum* *J Bacteriol 66* 34-40 1953

From 1935 through 1957 there has been a steady although fluctuating increase in the number of cases. In 1937 there was seen the first non fatal case and the ratio of fatal to non fatal has continued to increase through the years. In the last 5 years 156 cases have been reported of which 128 or 82% were non fatal and 28 or 18% fatal. While it may well be argued that many cases are not reported and this is unquestionably true, it is quite evident that the recognition of the disease is increasing. Whether this is due to an actual increase in the number of cases or increase in recognition is not readily apparent although the latter appears to be the case since the finding of cases appears to be directly related to the evidence of interest on the part of clinicians in any local area. This is clearly demonstrated by the fact that Chicago, Kansas City, Memphis and Nashville which are centers for the study of histoplasmosis report cases with the greatest frequency.

The age distribution of 490 reported cases is shown in Fig. 20. It is seen that the greatest frequency of cases and fatalities occurs in the age group under 1 year and 46 65 years. It seems possible that

TABLE V

INCIDENCE OF HISTOPLASMOSIS WITH RESPECT TO AGE AND SEX IN U. S. AND FOREIGN COUNTRIES FROM 1905 TO 19[illegible]8

	White		*Negro*		*Total*
	Male	*Female*	*Male*	*Female*	
United States					
Under 10	42	41	2	3	88
Over 10	270	64	13	6	353
Total					441
Foreign Countries					
Under 10	1	3	3	3	[illegible]
Over 10	31	0	5	1	43
Total					[illegible]0

there is a different causation for these two peaks since the younger peak is probably due to fatalities associated with first infection while the latter peak is probably due to endogenous reinfection

In Table V are shown the reported cases by race and sex as well as by place of residence of the patient It is seen that 121 of 491 cases were females or 25% and only 13 were non white Of the 491 cases only 50 or 10% were from foreign countries It is also seen that 80% of the United States cases and 86% of the foreign cases were over 10 years of age It is unquestionable that there are other areas of the world where histoplasmosis is extremely prevalent It appears also that the low percentage of reports from the foreign literature does not represent absence of cases in many areas but failure of recognition and reporting It is easy to predict not only increasing recognition of the disease in this country but in many foreign countries as knowledge of this most elusive fungus infection becomes more widespread

REFERENCES

1 Ingold C T *D sper al in Fungi* Clarendon Press Oxford England 19?3

Helmbright A L and Larsh H W The ze of the spores of *Histoplasma cap su at m Proc Soc Exper Biol M d 81* 0 51 19?

3 Larsh H W Scholes V E Hinton A an l S lberg S The minimal infectious inoculum of *Histoplasm capsulatum* for the mo se and chick embryo *Pr Soc Exper B ol & Med 9?* 5 0 5 3 19 ?

4 Cooke W B and Kabler P W The survi l of *H stoplas a c p ulatum* in water *P oc of the Confe ence on Histopl s 195* P b Health Monogr No 39 19?? pp ?61 ?64

Ritter C Studies of the vi bility of *Histopla a caf lat* in tap water *Am J Pub He lth 44* 1?99 1301 Oct 19?4

6 De Monbreun W A The cultivat on and c lt al characteristics of Darling s *Histopl sma capsulat m Am J T Med 15* 93 1? 1934

7 Brown J H Cook K M Nev F G a l Hatch T Influence of part cle size upon the retention of particle matter in the h uman lu g *Am J Pub Health 40* 450-4 9 19 0

8 Cozad G C and Furcolow M L Labo atory stu l es of *H plas caf sul tum* II S e of the Spores *J I f ct Di 9* 77 84 19 3

9 Grayston J T Altm n P I and Cozad G C E perimental hi toplasmos s in mice A prel mina report *Pr c f the Confe ence n Histopla mos s 195* Pub Health Mono No 39 19 6 pp 99 10?

10 Ajello L and Runyon L C I fect on of m e with single spores of *Hist plas ma caps lat m J Bact ol 66* 31 40 19 3

11 Emmons C W Rowley D A Olson B J Mattern C F T Bell J A Powell E and Marcey E A Histoplasmosis—Proved occurrence of inapparent infection in dogs cats and other animals *Amer J Hyg 61* 40 44 Jan 1955
12 Furcolow M L and Menges R W Comparison of histoplasmin sensitivity rates among human beings and animals in Boone County Missouri *Am J Pub Health 42* 926 929 1952
13 Emmons C W Isolation of *Histoplasma capsulatum* from soil *Pub Health Rep 64* 892 896 1949
14 Grayston J T Loosli C G and Alexander E R The isolation of *Histoplasma capsulatum* from soil in an unused silo *Science 114* 323 324 1951
15 Zeidberg L D Ajello L Dillon A and Runyon L C Isolation of *Histoplasma capsulatum* from soil *Am J Pub Health 42* 930 935 1952
16 Zeidberg L D Soil studies *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 240 250
17 Emmons C W The significance of saprophytism in the epidemiology of the mycoses *Tr New York Acad of Sc* Ser II *17* 157 166 1954
18 Ajello L Soil as natural reservoir for human pathogenic fungi *Science 123* 876 879 1956
19 Manos N E Ferebee S H and Kerschbaum W F Geographic variation in the prevalence of histoplasmin sensitivity *Dis Chest 29* 1 ?0 1956
20 Maddy Keith T Ecological factors possibly relating to the geographic distribution of *Coccidioides immitis* *Proc of the Symposium on Coccidioidomycosis held at Phoenix Ariz 1957* pp 144 157
21 Zeidberg L D A theory to explain the geographic variations in the prevalence of histoplasmin sensitivity *Am J Trop Med & Hyg 3* 1057 1065 1954
22 Zeidberg L D Ajello L and Webster R H Physical and chemical factors in relation to *Histoplasma capsulatum* in soil *Science 12* 33 34 1955
23 Emmons C W Histoplasmosis Animal reservoirs and other sources in nature of the pathogenic fungus *Histoplasma* *Am J Pub Health 40* 1950
24 Menges R W Furcolow M L and Hinton A The role of animals in the epidemiology of histoplasmosis *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 277 281
25 Schwarz J Baum G L Wang C J Bingham E L and Rubel H Successful infection of pigeons and chickens with *Histoplasma capsulatum* *Mycopathologia et mycologia applicata 8* 189 193 1957
26 Mochi A and Edwards P Q Geographical distribution of histoplasmosis and histoplasmin sensitivity *Bull Wo ld Health Organ 5* 259 291 1952
27 Gordon M A Ajello L Georg L K and Zeidberg L D *Microsporum gypseum* and *Histoplasma capsulatum* spores in soil and water *Science 116* 208 1952
28 Furcolow M L and Horr W H Air and water in the natural history of *Histoplasma capsulatum* *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 282 288
29 Zeidberg L D Dillon A and Gass R S Some factors in the epidemiology of histoplasmin sensitivity in Williamson County Tennessee *Am J Pub Health 41* 80 89 1951
30 Menges R W and Habermann R T Experimental Avian Histoplasmosis *Am J Vet Research 16* 314 320 1955

31 Becker I aul I ersonal commun cati n (U S Weather Bureau Dubuque Ia)

32 Edward I B Ieeples W J a d Berner A C I revalence of sensiti ity to tuberculin and histoplasmin among high sc l students in Montg mery Coun ty Maryland *Pediatrics* 1 3 9 3 C 19 9

33 Mattern C F T Bell J A Olson B J Emmons C W an l Powell F P *Proc of the C nference on H st pla osis 195* I ub Health Monogr No 39 19 6 pp 21 220

34 Emmons C W Campbell C C Hi t pla mosis in the D strict of Columbia Marylan l an l Vir inia *Clin P oc Child Hosp 13* No 11 pp 22 23 19 7

35 Emmons C W M rlan l H B an l Hill E L Hi to pla mos in rats and kunks in Ceorgia *P l Health Rep 64* 14 3 1430 1949

36 Aronson D L an l E lwards I Q An urb n focus of histo pla min sensiti ity *A Rev Tuberc & Pulm Dis* 9 85 80 19 9

37 I arrot T, Jr Tayl r G I oston M A, and Sm th D T An epidemic of his to pla mos s in Warrenton North Carolina *South M J 48* 114 1150 19 5

38 White F C Chronic pulm o ary disease i I t pla i n reactor A review of 229 cases I scovered thr gh chest clin c Examinatio *A er Rev Tuberc & Pulm D s* 2 1 29 19 5

39 Beadenkopf W C an l Loosli C C Hi topla mosis tuber ulosis a l coc cidio lomycosis *J A M A 146* 621 621 19 1

40 M s E The tornal as a epi lemi logical researcl t ol *Bull Ame Me I o olog cal Soc 39* 460 468 1 8

41 Kleinman H (Courtesy of Dr Kle nman State Department of Health University Campus M nneapolis 14 Minn)

42 Grayston J T Heeren R H an l Fur olow M I The geograph c distribution of histoplasmin re ctors am ng schc ol age cl l lren within a rural Iowa county *Am J Hyg 6* 201 213 19

43 Leh n P H and Furcolow M L Epi lem c l topla mosis *J Ch on Dis 5* 489 03 19

44 G ayston J T and Furcolow M I The oc urrence of histoplasmosis in epi lemics—Epi lemiol g al tu l es *An J Pub Health* Part I *43* 6 -6 6 19 3

45 Furcolow M L H stoplasmos s *GP 18* 11 127 1958

46 Fu colow M L and Ney Peter Epi lemiolog c a pects of histoplasmosi *Am J Hyg 65* 64 0 19 7

47 Anderson Col N W D to I L and Furcolow M L Cl ical x ray and serolog cal changes w th *H st plas* infect n *Pub Health Rep 73* 3 82 19 8

48 Sachs D Smith R ch I T Flem ng D S and F ircolow M L The prevalence of positi e react on t t iber ili an l hi topla in in a ural Minne sota County *A n J Hyg 6* 43 53 1

49 Chri t e A The d sease spect u of human h stoplas nos *A J t M d 49* 11 19 9

50 Straul M and Schwarz J P im y pulmonary arrested lesions of oc lio do mycos s and H stoplas nos s *Am J Cl n P th 26* 998 1009 19 6

51 Sasla S Maurice G E and Cole C R Experimental hi to lasmosis in la animals *J Lab & Cl n Med 46* (Abstract 96) 195

52 Straub M and Schwarz J The healed prim ry compl in h toplasmos s *A J Clin P th 5* 741 19

53 Furcolow M L Schwarz J Hewell B A and Grayston J T Incidence of tuberculin histoplasmin and blastomycin reactors among a group of school children *Am J Pub Health 43* 1523 1531 1953

54 Furcolow M L Guntheroth W C and Willis M J The frequency of laboratory infections with *Histoplasma capsulatum* Their clinical and x ray characteristics *J Lab & Clin Med 40* 182 187 1952

55 Ibach M J Larsh H W and Furcolow M L Isolation of *Histoplasma capsulatum* from the Air *Science 119* 71 1954

56 Palmer C E Nontuberculous Pulmonary Calcification *Pub Health Rep 60* 513 520 1945

57 Christie A and Peterson J C Pulmonary calcification and sensitivity to histoplasmin tuberculin and haplosporangin *J A M A 131* 658 660 1946

58 Aronson J D Saylor R M and Parr E I Relationship of Coccidioidomycosis to calcified pulmonary nodules *Arch Path 34* 31 43 1942

59 Sweany H C (Personal communication) Missouri State Sanatorium Mt Vernon Missouri

60 Loosli C G Histoplasmosis — Some clinical epidemiological and laboratory aspects *M Clin North America 39* 171 199 1955

61 Riley W A and Watson C J Histoplasmosis of Darling (with report of a case originating in Minnesota) *Am J Trop Med 6* 271 1926

DIAGNOSTIC PROCEDURES IN THE STUDY OF HISTOPLASMA CAPSULATUM

Howard W. Larsh

In recent years there has developed an enthusiastic interest in Histoplasma capsulatum and other systemic fungi which cause human disease. The number of diagnosed and reported systemic mycotic infections throughout the world increases many fold each year. Literature on the subject has become voluminous during the past ten years (1, 2).

Since 1950 the methodology used in the investigation of mycotic diseases has been improved and additional techniques have been added. Significant improvements in culture (3) and staining procedures (4-7) have been made during this period. The usefulness of animal inoculation in diagnostic procedures has been enhanced by additional investigations on methods of inoculation, size of inoculum and efficiency of adjuvants (8-22). Diagnostic methods which have proved useful in other biological and chemical studies have been modified and extended to the investigation of systemic mycoses. Tissue culture (23), polarized light (24), and fluorescent antibody (25) procedures have and will inevitably play important roles in advancing our knowledge of mycotic infections. Many of the recent epidemiological (26, 27), pathogenetic (28), and therapeutic (29-32) studies that have included the newer techniques have resulted in significant discoveries. It is these diagnostic methods that need further investigation so that they may be considered for wider adoption in future studies in this field of specialization.

CULTURE METHODS

In early mycological investigations (8), when plain Sabouraud's and other media were used, H. capsulatum was seldom isolated unless the clinical specimens received special handling. These media supported the growth of bacteria and contaminating sapro-

phytic fungi which grew much more rapidly than did the fungus It soon became evident that it was almost an impossibility to isolate the causal fungus from contaminated specimens although many ingenious methods were employed Today however with the addition of antibiotics to the various media encouraging results have been obtained but these procedures are not without failures The use of penicillin and streptomycin or chloramphenicol (33) to control the growth of bacteria and actidione (cycloheximide) (34) to retard the growth of saprophytic fungi has facilitated cultural studies The amount of each antibiotic added to the medium is dependent upon the source and type of specimen to be cultured In our experience media containing 10 000 units of penicillin 10 000 units of streptomycin and 1½ mg of actidione per ml have been useful in isolating H capsulatum from sputum and gastric specimens Biopsied and other aseptically obtained clinical specimens may be cultured on media containing lesser amounts or none at all Currently the combination of chloramphenicol and actidione is being used in an isolation medium These two antibiotics are relatively heat stable and can be autoclaved in the medium at 118°C (12 lb steam pressure) for 15 minutes This combination is available in commercially dehydrated media * Media containing antibiotics have been useful in obtaining isolations of H capsulatum and other pathogenic fungi from clinical and environmental specimens Unfortunately however clinical material containing a large number of cells of Candida and Geotrichum species will interfere with the isolation of Histoplasma capsulatum and other systemic fungi even on artificial media containing antibiotics In these cases the specimens may be treated with antibiotics and inoculated into experimental animals Failure to isolate the causal organism frequently occurs even with these procedures

Unequivocal identification of the H capsulatum sometimes requires that the tissue phase be observed This may be accomplished by animal inoculation of the clinical material followed by culturing tissue from sacrificed animals on artificial media Inoculated plates or tubes should be incubated at 37°C at a high

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Mycosel Agar Baltimore Biological Laboratory Inc., Baltimore Maryland. Mycobiotic Agar Difco Laboratories Detroit Michigan

humidity Histopathological verification can be made from stained tissues as well

The tissue phase also may be obtained by conversion from the mycelial phase directly on artificial media These include Francis glucose cystine blood agar (35) brain heart infusion glucose blood agar (36) Salvin s synthetic (37) Kurung s egg (38) and Pittman s liver spleen media (39) Although these media have proved successful in converting and maintaining H capsulatum the tissue culture technique is very useful for these procedures (40) In this method mycelial fragments are added to HeLa cell cultures and transferred to new cultures periodically until the conversion takes place The HeLa cell cultures are rotated in a roller drum similar to the procedure followed in studying some viral agents Once the fungus has been converted the HeLa cells are not needed for the maintenance of the tissue phase

HeLa cells show active phagocytosis and subsequent intracellular growth of H capsulatum (41) This would appear to provide another laboratory method for the experimental study of H capsulatum in human cells in tissue culture Some of the preliminary results indicate that HeLa cells in a specific tissue medium may be useful in the diagnosis of histoplasmosis However the usefulness of this technique for diagnosis of systemic mycoses must await the results obtained with clinical material now under investigation

CULTURAL CHARACTERISTICS

The diphasic nature of H capsulatum necessitates the presence of different chemical and physical environments for growth and identification of each phase Cultural characteristics of the yeast and mycelial phases vary on different types of media and with temperatures of incubation

On Sabouraud s dextrose agar at room temperature the fungus usually forms a white cottony aerial mycelium As the culture ages the mycelium may become buff to brown Most isolates of H capsulatum grow comparatively slowly requiring at least 10 to 14 days incubation for the production of a distinct colony (Fig 1*)

The assistance of Drs George C Cozad and Yousef Al Doory in the preparation of the illustration is gratefully acknowledged

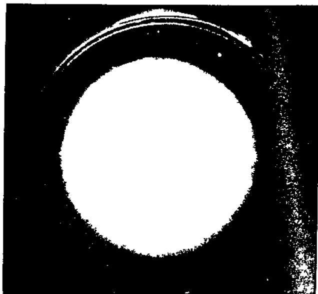


Fig 1 Histoplasma capsulatum on Sabouraud s dextrose agar 21 days at room temperature

It is imperative however to be cognizant of the fact that many isolates do not produce typical colonies During the past five years several isolates from patients with chronic histoplasmosis and on occasions from those receiving treatment with fungicidal agents produced colonies that may be designated as variants It is this type of an isolate that may be discarded without a positive identification of the etiological agent being made This is especially true since most of these isolates fail to develop tuberculated macroconidia (chlamydospores)

The mycelial phase from a typical colony consists of small delicate septate branched hyphae These structures may bear laterally sessile or stalked round to pyriform microconidia which measure 2 to 3 microns in diameter (Fig 2) Most of the microconidia are smooth but a few may be echinulated It is this phase of the mycelial growth that may be confused with other systemic or saprophytic fungi However later microscopical examination of most cultures will reveal large round or pyriform macroconidia These spores are much larger than are the microconidia (7 5 to



Fig 2 Histoplasma capsulatum from Sabouraud's dextrose agar Microconidia x700

18μ in diameter) and have a thickened spore wall (Fig 3) These macroconidia or chlamydospores may be smooth or tuberculated

Tuberculated macroconidia have been described as diagnostic or characteristic of H capsulatum Unfortunately they are not produced by all isolates of this fungus Cultures from several clinical specimens have not produced macroconidia These isolates have been grown on many types of media and observed on slide cultures Extensive microscopical examinations have failed to locate tuberculated macroconidia over a two year period of observations

Aerated liquid cultures of H capsulatum produce small flocculose to spherical forms of growth dispersed throughout the medium Microscopical examination of these growths show branching hyphae with little or no production of conidia (Fig 4)

The yeast phase may be obtained by inoculating white mice with the mycelial phase Three to six weeks after inoculation the mice are sacrificed and tissue from the reticulo endothelial system aseptically ground and planted on artificial media The inoculated media should be sealed and incubated at 35 to 37°C Within three to six days moist dull white colonies usually develop (Fig 5) on most yeast phase media In addition to animal inoculations the yeast phase may be obtained by converting the mycelial phase on

various artificial media Yeast colonies developing from the initial mycelial inoculum may be membranous and cerebriform They contain considerable mycelium and may be buff to reddish brown Successive transfers onto fresh media at intervals of three to five days may result in the development of moist yeast phase colonies Unless the cultures are incubated at low temperatures (4°C) it is



Fig 3 a *Histoplasma capsulatum from Sabouraud's dextrose agar Macroconidia* x700 b Single macroconidium showing tuberculations x700





Fig. 1 a Histoplasma capsulatum growing in Sabouraud's dextrose broth shake culture 21 days at room temperature b Mycelial preparation from growth in shake culture x700



Fig 5 Histoplasma capsulatum yeast phase on blood agar four days at 37 C

necessary to transfer them to fresh media every four or five days to maintain viable cells

Two artificial media which have been used advantageously in recent years for the conversion and maintenance of H capsulatum are potato flour—egg (38) and liver—spleen glucose blood agar (39)

Kurung and Yegian reported that on potato flour egg medium the conversion of H capsulatum to the yeast phase was easily accomplished even when strains had been maintained in mycelial stage for long periods They were successful in many instances in obtaining characteristic yeast like colonies on the first transplant from the mycelial phase In addition the yeast phase remained viable on potato flour egg medium for more than nine months

when incubated at 4°C and for at least three months when incubated at room temperature

Littman's liver spleen glucose blood agar is relatively efficient in the development of yeast phase growth of the diphasic fungi. The conversion of H. capsulatum to its yeast phase occurred in the first transplant from mycelium in most instances at 37°C. The systemic fungi can be maintained for long periods of time on this medium by submerging the growth with sterile mineral oil and incubating in the refrigerator.

Microscopically the yeast phase of H. capsulatum consists of small budding cells. They are predominantly ovoid and may measure from 1 to 5 microns in diameter (Fig. 6).

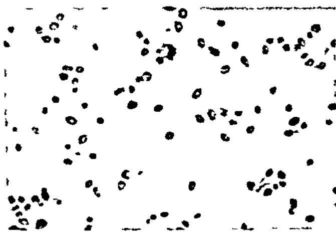


Fig. 6. Yeast cells from blood agar culture x ,00

ANIMAL EXPERIMENTATION

A. Experimental Histoplasmosis

Experimental histoplasmosis has been studied in many different species of animals including the white and brown mouse, guinea pig, white rat, albino rabbit, dog, hamster, rhesus and cynomolgous monkey.

The most extensive studies have been with the yeast phase of the fungus by intraperitoneal, intracerebral and intravenous routes

of inoculation Varying results have been obtained depending on the size of the inoculum and route of inoculation

Susceptibility of the white mouse to experimental histoplasmosis was shown by DeMonbreun in 1934 (8) and Parsons in 1949 (9) Infections resulted for inoculations of either the mycelial or the yeast phase Later Tager and Liebow learned that 3 to 4 weeks old white mice died of histoplasmosis 100 to 150 days after intraperitoneal inoculation of the mycelial phase (10) In 1945 Levy inoculated intravenously 6 to 8 weeks old white mice with a mixture of the yeast and mycelial phases (11) He was successful in isolating the fungus from the liver and spleen of all of the experimentally infected mice

Howell *et al* (12) showed that DBA line I mice were uniformly susceptible to the yeast phase of H capsulatum when inoculated intracerebrally Nearly all of the inoculated mice of this line died prior to thirty days after receiving 20 000 or more yeast cells The yeast phase proved to be highly virulent by this route of inoculation since 50% of the mice died between the seventh and tenth day following inoculation In a later publication Howell and Kepkie (13) reported that male DBA line 1 mice were relatively resistant to intravenous injections of the yeast phase of H capsulatum Peritoneal injections of large doses of saline suspended yeast cells also produced only an occasional death whereas a comparable number of cells suspended in 5% mucin increased the death rate in this strain of mouse Nevertheless the intracerebral route was far superior to the intravenous or intraperitoneal routes in producing experimental histoplasmosis

Campbell and Saslaw (14) have shown that white mice inoculated intraperitoneally were highly susceptible to the yeast phase of H capsulatum when 5% mucin was used as an adjuvant Approximately 79% of the mice inoculated with a standard inoculum in mucin died within thirty days after the injection Whereas in similar experiments less than 3% of the mice succumbed in 30 days when the diluent was saline instead of 5% mucin

Middleton McVickar and Peterson (15) were successful in producing experimental histoplasmosis in the Sherman strain of the white rat These investigators determined that the I D ranged

between 17 and 21 million viable yeast cells per rat when inoculated intravenously. The intravenous route, when suitable number of cells were used, yielded a consistently reproducible fatal infection in rats. However, intraperitoneal inoculation with the same number of heat-killed or living cells showed the rat to be highly resistant to this fungus in that no pathological effect was apparent.

Hazen and Tahler (16) produced experimental histoplasmosis of the skin and mucous membranes of the albino rabbit by inoculating with 2 ml of a 1:25 saline suspension of yeast cells intravenously. However, seven of the fifteen rabbits infected recovered completely two to three months after intravenous injection.

Farrell *et al* (17) showed that dogs could be experimentally infected by H. capsulatum. In dogs that were inoculated intratracheally with the yeast phase, acute progressive fatal experimental histoplasmosis resulted. When a similar dose of the inoculum was given by stomach tube to a series of dogs, no clinical disease occurred. The mycelial phase of H. capsulatum failed to produce clinical disease in dogs receiving the inoculum intragastrically or intratracheally. These investigators found cortisone-treated dogs susceptible to the mycelial phase when inoculated intratracheally but not by the intragastric route.

Salvin's (18) studies show that young hamsters inoculated intraperitoneally were more susceptible to small inocula of H. capsulatum yeast phase than were young guinea pigs or white mice. However, the use of 2.5% mucin as an adjuvant lowered the infectious dose in mice.

Drouhet and Schwarz (19) have shown the golden hamster to be more susceptible to the yeast phase of certain isolates of H. capsulatum when inoculated intraperitoneally than is the white mouse.

Hill and Marcus (20) were unable to isolate H. capsulatum from cynomolgous (M. irus) monkeys inoculated intratracheally and intracardially with the yeast phase. Animals inoculated intracardially produced serological titers and three were skin test positive but all specimens cultured failed to yield the fungus. These findings do not agree with the earlier work of DeMonbreun (8) who inoculated two rhesus monkeys (Macacus rhesus) intravenously

with the yeast phase of the fungus Both monkeys responded by having clinical and pathological signs consistent with histoplasmosis However one rhesus monkey failed to respond to an intravenous inoculation of the mycelial phase

Grayston and his co workers (21) exposed white Swiss mice intranasally to the mycelial phase by instilling droplets of the inoculum on the nose of lightly anesthesized animals Infection resulted from a small number of viable spores and the use of larger doses did not accelerate the pathogenesis of the disease Histopathological studies revealed little or no apparent reaction of the lung tissue to the inoculated spores It was evident that the lungs did not act as a serious barrier since the fungus was isolated from tail bleedings shortly after the injections

During the past few years in our laboratory intranasal experiments have been under investigation using the Henderson aerosol apparatus Exposure to aerosolized yeast and mycelial elements in the Henderson tube more nearly simulates the manner by which the infection must be contracted in nature Only the nose of the experimental animal is exposed for a definite period to the aerosolized inoculum

The results of these preliminary observations are most encouraging in that guinea pigs (Hartley strain) converted from a negative to a positive histoplasmin skin test when exposed to formalized inocula Guinea pigs receiving small doses of living inocula converted from a negative to positive skin test and many of them had detectable complement fixation antibody titers

B Animals in Diagnosis of Histoplasmosis

In many instances clinical specimens must be inoculated into laboratory animals for the isolation of the fungus and diagnosis of histoplasmosis Clinical specimens should be treated with antibiotics prior to injection into the animal Caution should be taken however not to include streptomycin in the specimens This antibiotic has proved to be extremely toxic to white mice in our investigation

To enhance the chance of isolating the fungus clinical specimens should be treated with an antibiotic solution for a long period of time Excellent results have been obtained when the specimen

and antibiotic solution were shaken vigorously with a paint shaking machine This mixture may then be inoculated intraperitoneally into mice Although the white mouse is not in every case infected with H capsulatum the species is an excellent tool in recovering the fungus from contaminated specimens There is considerable disagreement on the optimal time after inoculation to sacrifice the mouse The suggested period ranges from two to eight weeks In our experience the white Swiss mouse will seldom succumb to histoplasmosis within thirty days from an intraperitoneal inoculation Nevertheless when these mice are sacrificed and their tissue ground and plated on artificial media the fungus will be isolated Heavily contaminated specimens such as soil may result in bacteremia and death of the animal even if the specimens have been treated with antibiotics and daily parental inoculations of the mice followed Specimens from patients which contain an overwhelming number of Candida cells often destroy the animal before H capsulatum infection ensues

The great number of mice which were destroyed by environ mental specimens which had been treated with antibiotics prior to inoculation prompted a search for more efficient techniques of recovering pathogenic fungi from such specimens The cost of ani mals and antibiotics is high and the time required to adequately process a sample is a disadvantage In view of this a direct exposure of mice to soil and similar specimens was undertaken Also such a procedure would approximate a natural infection since man and animal probably become infected by inhaling aerosolized inoculum

The results of preliminary studies by Hinton *et al* (22) indi cate that the direct exposure method is more efficient than the indirect method of isolating H capsulatum from environmental specimens The mice do not have to combat bacteremia by this methodology Mortality rates were lower in the direct exposure method and the procedure requires less time and equipment This method is not infallible and certain precautionary measures are absolutely necessary The specimens should be completely dry which more or less assures that aerosols will be produced by the activity of the mice Failures have occurred because of leakage of water from the drinking bottles which ruined the sample How

ever in artificially seeded soil cultures and with soil collected from an area where humans were known to have been infected white mice became infected after exposure Few mice succumb to the infection within thirty days but should be sacrificed and tissue from the reticulo endothelial systems ground and planted aseptically on Sabouraud s glucose agar In addition to H capsulatum Cryptococcus neoformans Blastomyces dermatitides and Blastomyces brasiliensis have been isolated from mice exposed by the direct method on artificially inoculated soils Cryptococcus neoformans has been isolated from naturally infected specimens

Although excellent artificial media are available to the investigator animal inoculation still plays a major role in diagnostic medical mycology

STAINING METHODS

Within the past few years many new staining and microscopy procedures have been incorporated into the protocols of systemic mycotic investigations The earlier stains usually resulted in the detection of the etiological agents after long periods of searching The parasites can be observed in hematoxylin and eosin stained tissues The special stains of today however are much more useful especially in old chronic mycotic infections The periodic acid Schiff (Hotchkiss and McManus) (4) and Gridley (5) staining procedures have seen extensive use in recent years These special staining procedures are not infallible and often the fungus is difficult to delineate In the periodic acid Schiff method many carbohydrates and mucoproteins exhibit a staining reaction similar to that of the fungi Often only an experienced pathologist can make a positive identification of fungal elements A similar criticism can be made of the Gridley stain in that mucin and elastic fibers stain a deep blue as does fungus mycelium However the tissue phase of most of the systemic fungi can be identified as rose or purple stained structures by using the Gridley method At the present time the staining procedure of choice in many laboratories is the Gomori s methenamine silver nitrate method as adapted and applied by Grocott for staining fungi (6) This special staining

procedure has proved exceedingly helpful in revealing the parasites of histoplasmosis blastomycosis coccidioidomycosis and cryptococcosis It certainly would seem to be the procedure of choice in observing old chronic cases of mycotic infections In cryptococcosis Mayer s mucicarmine stain (7) is a useful method in studying the fungus in tissue sections In one or two cases studied by us this was the only stain that unequivocally revealed the parasite

POLARIZED MICROSCOPY METHOD

The adaptation of polarized microscopy to the study of mycotic infections is a relatively recent and interesting adventure Potenza and Feo (21) have shown clearly that fungal elements in clinical specimens were optically active Luminous areas were apparent in material from human mycoses and experimental mycotic lesions in animals The pathogens are optically active in unstained as well as stained tissues Unfortunately many intracellular inclusions in the systemic fungi also are birefringent Nevertheless one can usually learn to delineate these bodies from fungus material The fungi appear as colorless bodies whereas many of the other birefringent materials are colored

In our experience excellent results have been obtained by using the polarizing microscope on tissues from human cases of histoplasmosis stained with hematoxylin and eosin periodic acid Schiff and methenamine silver nitrate Tissues from Swiss white mice experimentally infected with Coccidioides immitis Blastomyces dermatitidis Cryptococcus neoformans and Histoplasma capsulatum have been observed with polarized light Characteristic birefringent forms were found in nearly all of the tissues examined revealing typical maltese crosses

It has been my good fortune to work with polarized microscopy under the tutelage of Dr Henry Sweany and his associates at the Missouri State Sanatorium These investigators have shown the polarized light technique to be an excellent aid in revealing the yeast phase of H capsulatum in chronic human histoplasmosis In several cases histopathologic diagnosis was made before the fungus was isolated from the tissue

The adaptability of polarizing filters and discs to almost any type of laboratory microscope makes this a rapid and easy procedure In addition the rapidity by which tissue sections can be scanned by the polarized light technique more than justifies its use in systemic mycotic investigations This technique should not be considered as a replacement for proved staining procedures but should be accepted as another aid in the diagnosis of mycotic infections

FLUORESCENT ANTIBODY METHODS

One of the most recent techniques that is proving useful in the diagnosis of diseases caused by many different agents is the fluorescent antibody method Coons *et al* in 1942 developed the technique but the methods have been extensively used in the last few years (25) A detailed description of the procedures of the methods were published by Coons and Kaplan in 1950 (42) The technique has proved very useful in the study of bacterial and viral diseases During the past year preliminary studies have been under investigation attempting to determine the usefulness of the fluorescent antibody methods in detecting H capsulatum in clinical specimens An evaluation of the direct and indirect fluorescent antibody methods as aids in the diagnosis of mycotic infections will have to await further investigations However Vogel and Padula (13) in a very recent publication showed that antibodies in sera with systemic mycoses were demonstrable by the indirect method

Improvements and simplifications in the procedures during recent years should stimulate additional studies to ascertain the usefulness of the fluorescent antibody technique in the diagnosis of mycotic infections

SUMMARY

It would appear that mycological investigations will become more efficient and greatly extended in view of the improved and recently added diagnostic laboratory methods Therefore it is imperative that all of the tools available for the isolation of the etiological agents and the diagnosis of the mycotic diseases be in

cluded in future studies Only through carefully planned and exhaustive studies will the real significance and importance of the fungal infections be determined Mycological as well as bacterio logical or viral studies should be included in all cases of so-called peculiar unusual or undiagnosed disease processes To accomplish these goals it should not entail too much additional media space or work for the investigators The results may be even more rewarding than they have been in the past few years

REFERENCES

1 Mochi A and Edwards P Q Geographical distribution of histoplasmosis and histoplasmin sensitivity *Bull World Health Organ* 5 259 291 19 ?

2 Procknow J J and Loosli C G Treatment of the deep mycoses *AMA Arch Int Med 101* 765 80 1958

3 George L K Ajello L and Gordon M A A selective medium for the isolation of *Coccidioides immitis Science 114* 38 389 19 1

4 Kligman A M Mescon H and DeLamater E D The Hotchkiss McManus stain for the histopathologic diagnosis of fungus diseases *Am J Clin Path 1* 86 91 1951

5 Gridley M F A stain for fungi in tissue sections *Am J Clin Path* 23 303 307 1953

6 Grocott R C A stain for fungi in tissue sections and smears using Gomori s methenamine silver nitrate technic *Am J Clin Path* 5 9 9 9 1955

7 Mayer s Mucicarmine Stain Mallory F B *Pathological Technique* Philadelphia Saunders 1942 p 130

8 De Monbreun W A The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum Am J Trop Med 14* 93 1? 1934

9 Parsons R J Experimental histoplasmosis in mice *Arch Path* 34 229 239 191

10 Tager M and Liebow A A Observation on histoplasmosis Induced infection in the mouse *Yale J Biol & Med 14* 469 430 1942

11 Levy B M Chemotherapy of experimental histoplasmosis in white mice *Am J Trop Med* 25 241 251 1945

12 Howell A Jr Kupkie G F and Bruyere P T Studies on experimental histoplasmosis *Public Health Rep* 65 722 735 19 0

13 Howell A Jr and Kupkie G F Experimental histoplasmosis Susceptibility of male DBA line one mice by various routes of injection *Proc Soc Exper Biol & Med* 75 121 123 1950

14 Campbell C C and Saslaw S Use of mucin in experimental infections of mice with *Histoplasma capsulatum Proc Soc Exper Biol & Med* 73 469 472 1950

15 Middleton J C McNickar D L and Peterson J C Experimental histoplasmosis in the white rat *Proc Soc Exper Biol & Med* 75 164 166 1950

16 Haven E L and Tahler E D Experimental histoplasmosis of skin and mucous membranes in rabbits *J Invest Dermat* 15 05 214 19 0

17 Farrell R L Cole C R I rior J A and Saslaw S Experimental histoplasmosis I Methods for production of histoplasmosis in dogs *Proc Soc Exper Biol & Med 84* 51 54 1953

18 Salvin S B Cultural and serologic studies on nonfatal histoplasmosis in mice hamsters and guinea pigs *J Infect Dis 94* 22 29 1954

19 Drouhet E and Schwarz J Comparative studies with 18 strains of histoplasma morphology in tissues and virulence of African and American strains *J Lab & Clin Med 47* 128 1 9 1956

20 Hill G A and Marcus S Challenge of *Macacus iris* with *Histoplasma capsulatum* *Am Rev Tuberc & Pulm Dis 75* 849 851 1957

21 Grayston J T Altman I L and Cozad G C Experimental histoplasmosis in mice *Pub Health Monogr* No 39 1956 pp 99 105

22 Hinton A Larsh H W and Silberg S L Direct exposure of mice to soils known to contain *Histoplasma capsulatum* *Proc Soc Exper Biol & Med 94* 1/6 1/9 1957

23 Larsh H W Hinton A and Silberg S L Conversion and maintenance of *Histoplasma capsulatum* in tissue culture *Proc Soc Exper Biol & Med 93* 612 615 1956

24 Lotenza I and Feo M Use of polarized light in diagnosis of mycotic infections *Tech Bul Am Soc Clin Pathologist 6* 91 99 1956

25 Coons A H Creech H J Jones R N and Berliner E The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody *J Immunol 45* 159 170 1942

26 Furcolow M L Recent studies on the epidemiology of histoplasmosis *Ann New York Acad Sc 7* 127 164 1958

27 *Proc of Symposium on Coccidioidomycosis (Section II) Held at Phoenix Arizona—Feb 11 13 1957* U S Dept of Health Education and Welfare Pub Health Service Bureau of State Services CDC Atlanta Ga

28 Procknow J J Page M I and Loo li C G Early pathogenesis of histoplasmosis Presented at NTA Annual Meeting May 18 23 1958 Philadelphia Pa

29 Lehan P H Yates J L Brasher C A Larsh H W and Furcolow M L Experiences with the therapy of sixty cases of deep mycotic infections *Dis Chest 3* 597 617 19 7

30 Harrell E R North American blastomycosis Treatment with amphotericin B Presented at NTA Annual Meeting May 18 23 1958 Philadelphia Pa

31 Salkin D Coccidioidomycosis Treatment Presented at NTA Annual Meeting May 18 23 1958 Philadelphia Pa

32 Rubin H Lehan P H FitzPatrick M J and Furcolow M L Amphotericin B in the treatment of cryptococcal meningitis *Antibiotics Ann 1957 1958* New York Med Encyclopedia Inc 19 8 pp 71 74

33 Ajello L Cultural methods for human pathogenic fungi *J Chron Dis 5* 54 5 1 May 19 7

34 Whiffen Alma J The production assay and antibiotic activity of actidione an antibiotic from *Streptomyces griseus* *J Bact 56* 283 291 1948

35 Campbell C C Reverting *Histoplasma capsulatum* to the yeast phase *J Bact 54* 263 264 1947

36 Howell A Jr Studies of Fungus Antigens I Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs *Pub Health Rep 6* 631 6 1 1947

3 Salvin S B Cysteine a l related ccmr un ls in the growth of the yea t l ke phase of *Hi toplasma capsulatum* *J Infect Dis* ""‚ "83 1949

38 Kurung J M and Yegian D Medium for Maintenance and Conversion of *His toplasma caf latum* to Yea tlike Phase *Am J Cl n Path* f ‚0 08 19 1

39 Littman M L Liver pleen glucose blood agar for *H stoplasma capsulatu* and other fungi *Am J Cl n Path* 5 1118 1159 1

40 Larsh H W Silberg S L and Hinton A Use of the tissue culture method in evaluat ng antifungal agents *Antib otics An al 1956 1957* New York Med Encyclopedia Inc., 19 pp 918 9""

41 Larsh H W an l Shepard C C HeLa cells and *H stoplasma capsulatum* pha gocytosis and subsequent intracellular growth *J Bact* 6 5 7 565 1958

42 Co ns A H and Kaplan M H Localization of antigen in ti sue cells II Im provements in a method for the detection of ant gen by means of fluorescent antibody *J Exper Med 91* 1 13 1950

43 Vogel R A and Padula J F Indirect stai ing reaction with fluorescent anti body for detection of antil sl es t pathogenic fungi *Proc Soc Exper Biol & Med 98* 135 139 19 8

THE SEROLOGY OF HISTOPLASMOSIS

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INTRODUCTION

Serologic methods for the study of histoplasmosis have contributed to the present knowledge of this disease Significant serologic studies independently were described in 1947 and 1948 (25 34 35 50) At that time the current status of histoplasmosis being a relatively common and usually benign entity rather than an uncommon and usually fatal disease still was indeterminate In large part this debatable status was attributable to the paucity of cases proved by culture isolation of the causative organism Reported proved cases were uncommon despite extensive efforts to isolate the organism from critically ill patients and from fresh surgical and autopsy material Histologic examination had yielded only sporadic evidence of Histoplasma like organisms Benign and brief acute pulmonary disease was unrecognized as a form of histoplasmosis Investigation of skin test converters (persons changing to a positive histoplasmin skin test from a previous negative skin test) with and without clinical illness by fungus culture methods presented a formidable and unrewarding laboratory chore Thus the thesis of histoplasmosis being a common disease depended upon the tenuous circumstantial evidence of skin test data described elsewhere in this volume More proved cases were needed for this thesis to be valid This then was the climate of knowledge of histoplasmosis when serology seriously became investigated

The serologic tests to be described furnished a tool for case finding They have served as a survey screening method which has led to significant numbers of proved cases Also they have aided in the understanding of the epidemiology of histoplasmosis by furnishing additional circumstantial diagnostic evidence Today when the thesis that histoplasmosis is a common and usually benign disease no longer seriously is contested these serologic methods

afford an important practical aid in the clinical diagnosis of histoplasmosis

Facets of serology which seem appropriate for this discussion include (1) the various tests available (2) the sensitivity and specificity of the tests with emphasis on comparative studies (3) significant serum titers and their duration (4) serologic patterns of disease (5) cross reactions (6) variations in antigenic preparations (7) antigenic fractions (8) the relationship of serology and the skin test and a summary of practical implications A presentation of representative data rather than a complete bibliographic review is intended

I The Serologic Tests

The tests studied utilize the serologic principles of (a) complement fixation (b) agglutination and (c) precipitin reactions The various complement fixation tests differ from one another both in technic (quantity of reagents conditions of incubation criteria of fixation) and in the nature and concentration of the antigen The antigens consist of yeast phase products (whole yeast suspension ground yeast supernatant sonic disintegrated supernatant) on the one hand and mycelial phase products (histoplasmin or fractions of histoplasmin) on the other The agglutination tests (collodion particle agglutination hemagglutination latex particle agglutination) and precipitin test employ histoplasmin as the antigen—either as antigenic coating of particulate matter in the former tests or as a soluble antigen in the latter

A The Complement Fixation Tests 1 The Test of Salvin (25) employs formalinized whole yeast phase antigen With sera obtained from Histoplasma infected rabbits antibodies in high titer appeared within 2 weeks and persisted for 23 weeks In contrast histoplasmin as antigen detected antibodies over a briefer period 2 to 7 weeks and in lower titer Also Histoplasma yeast phase antigen gave no cross reactions with sera from Blastomyces infected rabbits whereas histoplasmin reacted in low titer with these sera

2 The Test of Tenenberg and Howell (50) employs histoplasmin as antigen With sera obtained from guinea pigs infected

with H capsulatum complement fixation was noted These sera were obtained seven or more months after infection Similarly blastomycin antigen detected complement fixing antibodies in sera from guinea pigs infected with Blastomyces dermatitidis Cross reactions between these antigens histoplasmin and blastomycin were noted when tested against the heterologous antisera However the homologous reactions were present in higher titer than were the heterologous reactions and relative specificity was demonstrable by the method of serial serum dilutions

Application of this test to human undiluted sera (10) revealed complete complement fixation in 14 of 300 different sera These positive tests were with the sera of 6 of 9 proved cases of histoplasmosis 2 of 13 recent skin test converters and 6 of 36 persons with radiologically unhealed pulmonary lesions and positive histoplasmin negative tuberculin skin tests None of 242 control sera gave complete complement fixation though 5 1% revealed partial fixation Cross reactions were noted with blastomycin antigen but the homologous antigen histoplasmin gave higher titers

3 The Test of Saslaw and Campbell employs whole yeast phase (35) and ground yeast phase supernatant (36) as antigens With sera from rabbits immunized with heat killed yeast phase H capsulatum complement fixation was noted with both these antigens Blastomyces yeast phase antigen fixed complement when tested with sera from rabbits immunized with Blastomyces dermatitidis Cross reactions were noted with Histoplasma and Blastomyces yeast phase antigens when tested with the heterologous antisera The homologous reactions were of higher titer Ground yeast phase Histoplasma antigen gave a greater degree of cross reaction than did the whole yeast phase antigen Because of the cross reactions of Histoplasma and Blastomyces yeast phase antigens and their respective heterologous antisera the authors recommended the inclusion of both these antigens in complement fixation tests for histoplasmosis

B The Agglutination Tests 1 The agglutination test studied most extensively is the Collodion Agglutination Test of Saslaw and Campbell (34) Collodion particles coated with histoplasmin are incubated with various dilutions of serum and macroscopic

clumping is the criterion of the presence of antibodies Employing sera from rabbits immunized with heat killed yeast phase Histoplasma organisms significant agglutination of the coated collodion particles was noted Significant serum titers (implied though not explicitly stated) ranged from 1 20 to 1 80 Blastomyces antiserum in low titer cross reacted with these particles Several different preparations of histoplasmin were found to be equally suitable as antigen (37)

In tests with human antisera (38) strong collodion agglutination reactions (1 20 to 1 80) were noted in 3 cases of acute histoplasmosis and weak to negative reactions (less than 1 5 titer) in 3 cases of chronic histoplasmosis The collodion agglutination titers paralleled yeast antigen complement fixation titers which ranged from 1 160 to 1 1210 in the acute cases and 1 20 or less in the chronic cases Weak to negative reactions with collodion agglutination and ground yeast phase antigen complement fixation again were noted in a blind study of human sera from chronic cases of histoplasmosis (40)

2 Other agglutination tests include (a) A histoplasmin latex agglutination test (42) similar in principle to the collodion agglutination test save for the use of latex particles instead of collodion particles Preliminary studies with animal (dog monkey and rabbit) and human antisera were similar to the results of collodion agglutination—titers were elevated early in the disease and became negative in 2 to 3 months during convalescence (b) A hemagglutination test (20) in which sheep erythrocytes are coated with histoplasmin Agglutination was noted with sera from Histoplasma immune rabbits A zone phenomenon (inhibition of agglutination) was noted in the presence of excess antigen

C* *The Precipitin Test of Salvin and Hottle (26) employs histoplasmin as antigen * Sera from rabbits infected with yeast phase Histoplasma capsulatum and hyperimmunized with formalin

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Histoplasmin and human sera are employed in precipitin reactions in agar gel Sensitivity and cross reactions with heterologous fungus antigens and antisera are described Different bands of precipitation are interpreted as distinctive of specific vs nonspecific reactions as well as distinctive of true vs false positive reactions (Heiner D C Diagnosis of histoplasmosis using precipitin reactions in agar gel *Pediatrics* 616 6 7 19 8)

killed organisms were tested with 4 Histoplasma antigen preparations—filtrate of ground yeast phase yeast phase broth culture filtrate ground mycelial phase filtrate and mycelial broth filtrate (histoplasmin) Significant titers appeared at 13 days and reached a peak at about 20 days Titers (highest dilution of antigen giving a precipitin reaction) were maximal and of longest duration (76 days) with histoplasmin

In studies of sera from proved human cases of histoplasmosis precipitin titers of 1 8 or greater appeared to be significant though chronologic change in titer seemed even more significant (30) Cross reactions with heterologous antigens (blastomycin and coccidioidin) were noted However precipitates with the homologous antigen usually were present with a greater antigen dilution and persisted for a longer period of time than with the heterologous antigens A zone phenomenon due to excess antigen was noted only very rarely

A brief summary of the preceding discussion of the available tests for histoplasmosis indicates that all of the available antigenic preparations and the in vitro techniques employed are effective with experimental animal and human antisera With a given serum the tests vary in sensitivity and specificity Cross reactions to be discussed further occur between Histoplasma antigens and heterologous antisera as well as between heterologous antigen and Histoplasma antisera

II Sensitivity and Specificity—Comparative Studies

It appears obvious that a test to be of practical value must possess a relatively high degree of sensitivity (detect a significant per cent of positive sera in an infected population) and specificity (fail to be positive in an uninfected population) The sensitivity of a test and consequently the titer obtained with it readily may be altered by varying the concentration of antigen and other reagents as well as the conditions of incubation and reading of the test An immunologic truism might read— increasing the sensitivity of a test jeopardizes its specificity so a compromise between sensitivity and specificity must be made if a test is to be practicable

A consideration of the tests previously discussed may convey the expectation that those employing histoplasmin as antigen would

yield parallel qualitative data since the same antigen is employed in each Accordingly the histoplasmin complement fixation collodion agglutination and precipitin tests would reveal comparable data However variations of sensitivity and specificity inherent in these different serologic technics as well as variations in the antigenic properties of different histoplasmin preparations introduce factors which contribute to observed discordant results Similarly tests employing yeast phase antigens may be expected to vary in comparative studies not only do different yeast phase preparations vary in potency but also whole yeast with an intact surface does not present the same antigenic complex as do the components of disrupted yeast cells Further as indicated earlier and to be discussed again later tests vary in sensitivity depending upon the stage of infection represented by the serum specimen to be tested

Inherent fundamental differences between complement fixation agglutination and precipitin reactions render quantitative comparisons (titers) misleading For example a zone of inhibition due to excess antigen is most common in the precipitin technic less likely in agglutination reactions and insignificant in complement fixation Also precipitin titers are incomparable with complement fixation and agglutination titers since in the precipitin test antigen rather than serum dilutions are employed In the precipitin test equivalence of antigen to antibody rather than antibody concentration is determined *increasing* titer reveals diminishing rather than increasing antibody concentration to the point of inadequate antigen for visible aggregation of particles The collodion agglutination test in which histoplasmin also is used as the antigen but in which serum dilutions are employed should on a theoretical basis be a more valid quantitative test for antibody concentration than is the precipitin test Ignoring serologic theory however the precipitin and collodion agglutination tests as herein discussed empirically have proved to be of value under similar circumstances (28 29 39 40)

Some comparative results of tests on a given serum already have been mentioned Salvin (25) with complement fixation tests employing serum from infected rabbits found whole yeast phase antigen superior to histoplasmin in the degree and duration of titer

Grayston (13 14) with complement fixation tests of human and rabbit antisera found whole yeast histoplasmin and ground yeast supernatant of diminishing sensitivity in this order Hill and Campbell (15) in complement fixation tests with human sera and whole yeast and histoplasmin antigens found that either antigen would have been diagnostic in many cases Both antigens however were necessary for maximal detection of diagnostic titers—whole yeast antigen reacted in higher titer with sera from cases of early primary pulmonary histoplasmosis whereas histoplasmin was more sensitive in chronic cases with radiologic evidence of pulmonary cavitation fibrosis or pleural effusion In the latter clinical group whole yeast phase antigen frequently failed to reveal a significant titer

Saslaw and Campbell (38) as discussed earlier found that collodion agglutination and yeast phase complement fixation tests gave high titer in acute human histoplasmosis and weak to negative reactions in chronic disease This is similar to the findings of Salvin and Furcolow (28 30) who found the precipitin test positive in early stages of histoplasmosis However the latter authors differ in their results with whole yeast phase antigen they found yeast phase complement fixation tended to be positive in the later stages of infection

Sabin (24) compared histoplasmin and whole yeast phase antigen in complement fixation with human sera from an epidemic of histoplasmosis He reported higher titers with whole yeast phase antigen and weak to negative titers with histoplasmin He concluded on the basis of this single study in which the same strain of H capsulatum presumably was the cause of all the cases that the superiority of the yeast phase antigen as a tool for the diagnosis of acute and recent histoplasmosis is quite apparent in these tests *This is quite in contrast with the findings of Salvin and Furcolow* (28 30) mentioned above as well as with the result of Rubin Lehan and Furcolow (23) who found whole yeast phase antigen complement fixation negative throughout the 3 month period of an acute case of histoplasmosis Histoplasmin complement fixation was positive early (23 days after the onset of illness) and became negative

3 weeks later (Precipitins were demonstrable with only one of the serial sera.)

In an extensive comparative "blind study" of different tests with human sera, Tenenberg (51) found whole yeast phase antigen more sensitive and more non-specific than histoplasmin antigen in the complement fixation test. With the same sera, Saslaw (40) found both the collodion agglutination and ground yeast phase antigen complement fixation relatively insensitive, whereas Salvin (29) found the precipitin test insensitive but whole yeast phase complement fixation sensitive and relatively specific.

An explanation of these apparent discrepancies appears to lie in the fact that many of the sera which were expected to be positive on the basis of having been obtained from proved and presumptive cases of histoplasmosis represented specimens obtained relatively late in the course of the disease. As indicated earlier, the precipitin and collodion agglutination tests tend to be positive early and transiently. Therefore evaluation of the sensitivity and specificity of the precipitin and collodion agglutination tests with "late stage" sera is misleading; the stage of disease represented by the sera must be considered. This consideration, however, does not explain the disparities between the results of Tenenberg (51) and Salvin (29) with whole yeast phase antigen and Saslaw (40) with ground yeast phase antigen. Perhaps the variation in antigenic properties of different lots of antigen contributes to the differences noted.

In a study of chronic progressive cavitary histoplasmosis, Lehan, Brasher, Larsh and Furcolow (18) found that the histoplasmin complement fixation test was slightly more positive and gave higher titers than the whole yeast phase test. The histoplasmin test was positive in 80% of cases, whereas both tests together serologically detected 90% of the cases. They conclude from this and other data that the precipitin test usually is positive earlier than the complement fixation tests and that neither histoplasmin nor whole yeast phase antigen alone give as high a degree of sensitivity as when employed concurrently. Their recommendation that tests with two (or more) antigens be employed for most effective detection of antibodies is similar to that of Hill and Campbell cited earlier.

Grayston (13, 14), with complement fixation tests of human and rabbit antisera, found whole yeast, histoplasmin and ground yeast supernatant of diminishing sensitivity, in this order. Hill and Campbell (15), in complement fixation tests with human sera and whole yeast and histoplasmin antigens, found that either antigen would have been diagnostic in many cases. Both antigens, however, were necessary for maximal detection of diagnostic titers—whole yeast antigen reacted in higher titer with sera from cases of early primary pulmonary histoplasmosis, whereas histoplasmin was more sensitive in chronic cases with radiologic evidence of pulmonary cavitation, fibrosis or pleural effusion. In the latter clinical group, whole yeast phase antigen frequently failed to reveal a significant titer.

Saslaw and Campbell (38), as discussed earlier, found that collodion agglutination and yeast phase complement fixation tests gave high titer in acute human histoplasmosis and weak to negative reactions in chronic disease. This is similar to the findings of Salvin and Furcolow (28, 30), who found the precipitin test positive in early stages of histoplasmosis. However, the latter authors differ in their results with whole yeast phase antigen; they found yeast phase complement fixation tended to be positive in the later stages of infection.

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IV Serologic Patterns

Serologic patterns (the sensitivity of different tests at various stages of infection) have been alluded to earlier The precipitin test (28 29 30) and collodion agglutination test (38 40) tend to demonstrate their highest titers early in the disease and for a transient period and are weak to negative in the later stages of infection Conversely the complement fixation tests with histoplasmin and yeast phase antigens tend to be positive in the later stages of infection (17 18 29 31) (Exceptions to these patterns have been noted (3 4 6 15 23 24)) These patterns are analogous to those in Coccidioidomycosis (18) in which the precipitin reaction was found to be most useful early in the disease (1 to 3 months) and the complement fixation test more useful in later stages (3 months to a year or longer)

An attractive hypothesis applicable to the relatively short lived status of the precipitin and collodion agglutination tests is that of Martin (19) He suggests that soluble antigen may combine in vivo with circulating antibody and an excess of antigen may be present The serum thus may provide insufficient free antibody for in vitro aggregation or clumping of particles Complement fixation still may occur since it is not necessary that a precipitate should form for complement to be fixed for this may occur in zones where there is no visible precipitation (1)

Campbell and Binkley (6) discussed three different disease patterns and their associated serology In cases of (a) primary acute pulmonary histoplasmosis they found that ground yeast phase complement fixation reached peak titers usually within the first 6 weeks of illness and rapidly declined by the fourth month In cases of (b) chronic generalized disease high titers for long periods (6 months or longer) were usual In (c) advanced generalized histoplasmosis low to negative titers were found

Negative serologic tests (2 13 18) as well as negative skin tests (2 18) have been described by others in chronic to fatal histoplasmosis It is not known whether this depression is specific or merely a reflection of a nonspecific depression associated with critical illness as noted in skin tests (11)

Salvin and Furcolow (30) commented upon the relatively early

III Significant Titers and Duration of Titers

The liberty has been taken to infer from published studies with human sera the titers of each test which appear to be significant Understandably the definition of a positive or significant titer generally has been avoided A titer per se may be misleading a low titer with a relatively less sensitive test may have greater significance than a high titer with a more sensitive test The sensitive test yielding high titers may at the same time give nonspecific or cross reactions to an extent that diminishes its value Emphasis has been placed upon the value of tests on serial serum specimens particularly when the serum titers are of equivocal interpretation The rationale is that a changing titer is a reflection of current or recent antigenic stimulus (infection)

With the complement fixation test of Salvin employing whole yeast phase antigen significant titers of 1 20 1 40 and a duration of titers for 5 weeks to 18 months (13) 3 weeks to 6 months (24) and up to 8 years (32) have been reported

The complement fixation test of Saslaw and Campbell with whole yeast phase antigen is considered suggestive in a titer of 1 10 to 1 80 and presumptive in a titer of 1 160 Significant titers have varied in duration from 2 to 16 weeks in acute cases and for 48 weeks or longer in chronic cases (6 15)

The complement fixation test of Saslaw and Campbell with ground yeast phase antigen may be considered positive in a titer of 1 10 to 1 160 and has persisted for 2 to 16 weeks or longer (3 6)

The complement fixation test of Tenenberg and Howell with histoplasmin antigen has been considered positive in a titer of 1 1 to 1 5 (2 10 51) With Schubert s modification of this test (43) a titer of 1 8 is considered significant (18) The duration of a significant titer has ranged from 3 to 8 weeks in acute (23) and from a few weeks to years in chronic (2 51) cases

The precipitin test has been considered significant in an antigen titer of 1 4 to 1 8 and may be positive from 1 to 2 weeks to 5 or 8 months (30) or longer

The collodion agglutination test has been considered significant in a titer of 1 20 to 1 80 and may persist from 2 to 3 weeks to 4 months or longer (31 38 39)

with Histoplasma antigens and antisera from rabbits immunized with C albicans C neoformans S schenkii and B brasiliensis The serologic cross reactions are similar to those observed in animal skin tests with histoplasmin blastomycin coccidioidin and haplosporangin (9)

In general homologous reactions have been distinguished from heterologous reactions by employing appropriate serum dilutions higher titers being obtained with the homologous antigen This specificity demonstrable by differential titrations is similar to that of the skin test Howell (16) demonstrated relative specificity with the homologous skin test antigens (histoplasmin and blastomycin) when the appropriate antigen dilution (critical titer) was employed

Tempering the generalization that homologous reactions are stronger than heterologous (cross) reactions are the findings of Campbell and Binkley (6) They found that ground yeast phase antigens of Histoplasma and Blastomyces gave extensive cross reactions in complement fixation tests with sera from cases of Blastomycosis and Histoplasmosis respectively Many of the cross reactions were indistinguishable from the homologous reactions by serum titrations Coccidioidin antigen and coccidioides human antiserum also cross reacted but to a less marked degree Because of these cross reactions antigens from each of these three fungi were recommended for routine serologic tests in these three diseases

The confusing problem of cross reactions raised by these studies of Campbell and Binkley is mitigated somewhat by the realization that *ground* yeast antigens were employed It has been shown that ground yeast phase Histoplasma gives greater cross reactions with Blastomyces antisera than does whole yeast phase antigen (36) Salvin even failed to find cross reactions with whole yeast Histoplasma antigen and Blastomyces rabbit antisera though he did find histoplasmin antigen cross reacted in low titer (25)

The problem of cross reactions appears to be significant but soluble if suitable titrations and the appropriate antigens are included in serologic testing The independent geographic distribution of endemic histoplasmosis and coccidioidomycosis further decreases confusion in serologic interpretation in the study of a

and transient appearance of significant precipitin titers in contrast with the more prolonged significant titers in the whole yeast phase complement fixation test They postulated that this type of data might be employed as a prognostic guide a negative precipitin and a positive complement fixation test indicating a poor prognosis This is analogous to the suggestion of Smith *et al* (46) who found in coccidioidomycosis that a pattern of a high and or rising complement fixation titer and a negative precipitin test was associated with the severity of infection Contrarywise Crayston (13) did not find complement fixation titers correlative with the severity of infection which is somewhat in agreement with Campbell and Binkley (6)

The data cited in the preceding three sections indicate that the precipitin and collodion agglutination tests tend to be positive in acute or early stages of chronic histoplasmosis whereas the complement fixation tests tend to be positive in the later stages or of relatively low titer in early stages of disease There is frequent overlap so that this generalization is not inflexible Thus two or more different serologic tests in combination are more sensitive than only one test

Though desirable prognostic serologic patterns have not convincingly been demonstrated Each serologic test can vary from positive to negative at all stages of infection

X Cross Reactions

Cross reactions represent an aspect of specificity Histoplasma antigens demonstrate cross reactions with other fungus antisera Conversely Histoplasma antisera demonstrate cross reactions with other fungus antigens Similar cross reactions involving the same fungi occur with skin tests Apparently these fungi are related antigenically

The most common cross reactions involve Blastomyces antigen and antisera and they occur in all the tests studied in this regard with both human antisera (2 6 10 15 18 30) and animal antisera (21 25 34 37 50) Similar reactions have been observed with Histoplasma and Coccidioides antigens and antisera (16 15 18) No cross reactions have been demonstrated in the collodion agglutination test (35) or whole yeast phase complement fixation test (36)

VII Antigenic Fractions

Despite the extensive use of histoplasmin and yeast phase products in skin testing and serology there has been relatively little study of purified fractions of these antigenic substances

The serologic properties of the crude supernatant of ground and sonic-disintegrated yeast phase Histoplasma have been mentioned earlier Salvin and Ribi (33) have presented evidence that the antigenic activity in complement fixation resides in the cell wall rather than in the cytoplasm of the yeast phase Histoplasma

Sorensen and Evans (19) isolated a protein free antigen (presumably polysaccharide) from the supernate of a fluid culture of yeast phase Histoplasma This fraction fixed complement when tested with sera from Histoplasma infected rabbits and did not cross react with sera from Blastomyces and Coccidioides infected rabbits This is similar to the findings of Pates (21) who isolated a polysaccharide like fraction of histoplasmin which gave a precipitin reaction with serum from Histoplasma inoculated rabbits and which did not cross react with serum from rabbits inoculated with Blastomyces In addition three other fractions—protein like polysaccharide like and of undetermined chemical nature—reacted not only with Histoplasma antisera but also cross reacted with Blastomyces antisera

It seems appropriate to mention the polysaccharide fraction of histoplasmin studied by Cross and Howell (8) which gave positive skin tests in Histoplasma and Blastomyces infected guinea pigs The homologous skin test reaction was greater than the heterologous reaction This polysaccharide immunologically is unlike the fraction of Sorensen and Evans and is similar to the polysaccharide like fraction of Pates which cross reacted with Blastomyces antiserum

A provocative study is that of Campbell (7) who investigated the complement fixing properties of ethanol precipitated crude fractions of both histoplasmin and ground yeast phase H capsulatum At least two antigenically different components were obtained one fraction precipitated by 0 1 mole ethanol and which predominated in the yeast phase complex and a second fraction precipitated by 0 4 mole ethanol and which was found in both the

stable population However the apparent overlap of the geographic distribution of histoplasmosis and blastomycosis (47) renders epidemiologic considerations less helpful in distinguishing these two diseases Also study of migrant cases is not clarified by *epidemiologic considerations when cross reactions are involved* So antigenic preparations from Histoplasma Coccidioides and Blastomyces may be needed in tests with certain sera

VI Variations in Antigenic Preparations

The antigenic properties of yeast phase and mycelial phase (histoplasmin) antigen vary depending upon the strain of H capsulatum from which they are derived Significant variations in potency of histoplasmin (14) and whole yeast phase (46) complement fixing antigens were found when comparing lots of each produced by several *different* strains of H capsulatum in tests with both human and rabbit antiserum (This is comparable to the variation in potency of different lots of histoplasmin in skin tests) (16) Further serum from rabbits immunized with a given strain *of Histoplasma did not necessarily give highest titers with antigens* from this same strain antigenic potency in serology varied independent of whether the test serum was homologous or heterologous to the strain of antigen This variation in potency is contrary to the findings of Salvin and Hottle who found no significant variation in the potency of histoplasmin produced by several strains of H capsulatum (27)

Comparison of histoplasmin and yeast phase antigens each derived from the *same* strain of Histoplasma revealed variable interpretations of potency depending upon the nature of the serum employed (45) Thus with hyper immune rabbit antiserum all three antigens tested (histoplasmin whole yeast phase cells and supernatant from sonic-disintegrated yeast cells) were of equal complement fixing activity *With serum from infected rabbits whole* yeast phase *antigen was inferior to the other two antigens* With serum from proved and presumptive human cases of histoplasmosis histoplasmin was the most reactive The authors conclude that antigens should not be preselected on the basis of tests with non human sera

negative serology became positive Similar case studies are described by Salvin and Furcolow (30)

The serologic tests in significant titer as described earlier are of variable duration The histoplasmin skin test once positive usually remains so for years to life long Thus in a followup study of the Camp Cruber Epidemic (17) more than 6 years after the epidemic all 26 cases studied still had positive skin tests but only 7 had positive serologic tests Other studies of epidemics have yielded similar data (12) Further evidence that skin tests are more persistent than the serologic tests is the observation that skin test converters usually continue to have positive skin tests thereafter whereas they usually have negative serologic tests when studied serologically (10 29 40 51)

Discordant results of skin tests and serology may occur during clinically active histoplasmosis either may be positive and the other be negative Thus positive skin tests and negative serology as well as negative skin tests and positive serology have been observed (10 17 52) The non specific depression of skin reactivity associated with critical illness (11) has not been shown to account for the depression of serologic reactivity

B Influence of Skin Testing Upon Subsequent Serology There is evidence that repeated skin testing with histoplasmin does not induce a positive skin test in humans (22 31 41) or in guinea pigs (16) Similarly repeated negative skin tests with histoplasmin fail to induce positive serologic tests in humans (22 31 41) or in guinea pigs (50) Further a single positive skin test fails to induce positive serologic tests (3 22 31 41) However frequently repeated positive skin tests at 1 to 2 week intervals have induced positive collodion agglutination tests (22 41) complement fixation tests (31 41) and precipitin tests (31)

It is unlikely that skin testing significantly influences the serologic results of clinical and epidemiologic studies such as those described in this chapter Repeated positive skin tests rarely if at all are performed at such short intervals as those mentioned above there is little if any indication for repeating a skin test if one has been positive a short time before Nevertheless since it has been shown that a positive skin test in an already skin test positive indi

yeast phase complex and histoplasmin The 0 1 mole fraction reacted with sera from early stages of histoplasmosis and cross reacted with serum from early coccidioidomycosis and cutaneous blastomycosis The 0 1 mole fraction was more concentrated in the histoplasmin and reacted with serum from late but not early stages of histoplasmosis it did not cross react with serum from cases of coccidioidomycosis or blastomycosis

Generalization from these data of Campbell should not be made because of the small number of antisera tested and because only one lot each of fractionated yeast phase and histoplasmin were studied Other lots of these antigens similarly fractionated might yield different results An intimation that this might be so lies in the observation cited earlier that histoplasmin has been shown to react with acute or early stage serum in the complement fixation test as well as in the precipitin and collodion agglutination tests Also yeast phase antigen has been shown to react with late stage serum

Further study of fractions of Histoplasma substances (yeast cells and histoplasmin) would be desirable Since these crude complexes represent antigenic mixtures purified fractions of them may yield more specific and possibly more sensitive antigens However the spectrum of observed antibody responses in histoplasmosis suggests that the crude antigenic complexes are more liable to detect the presence of one or more of the several species of antibody than would a purified antigen which reacts with only one species of antibody If this latter concept is valid with purified fractions as antigens even more tests than now are recommended would be necessary for adequate serologic screening and diagnosis

VIII Relationship of Serology and Skin Test

A Onset and Duration of Serology, Relative to Skin Test

In general the histoplasmin skin test becomes positive (at 2 to 3 weeks) before the serologic tests (at 3 to 4 weeks) following initial infection An excellent example of this is the case study of Rubin Lehan and Furcolow (23) in which the skin test was found to be positive 4 days after the onset of illness and 18 days after exposure to infection About 2 weeks after the onset of illness the initially

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influence upon serologic results Repeated skin testing at short intervals may influence serologic results in skin test positive individuals and should be avoided

(9) There is no reliable prognostic titer or pattern Negative to high titers may be obtained at all stages of infection with each test

(10) Standardized reagents are necessary for valid serologic results Serology is best performed in laboratories experienced with these tests

(11) Isolation of H capsulatum by culture methods is the most valid diagnostic criterion of histoplasmosis

REFERENCES

1 Boyd W C *Fundamentals of Immunology* Interscience 1943 p 2 ,

2 Bunnell I I and Furcolow M L A report on ten proved ca es of histoplas mos s *Pub Health Rep 63* 99 316 1948

3 Campbell C C and Saslaw S Use of yeast phase antigens in a complement f xat on test for histoplasmosis *Pub Health Rep 64* 551 560 1949

4 Campbell C C Use of yea t phase antigens n a complement fixation test for histoplasmos s IV Results w th ground yeast phase antigens in serial specimens of serum from thirty seven patients In Unit d States Department of Health Education and Welfare *P c of the Conference on Histopla mosis 1952* Pub Health Monogr No 39 19 6 pp 140 143

5 Campbell C C Cross reactions of mycotic ant gens *Ibid* pp 144 148

6 Campbell C C and Binkley G E Serol gic diagnosis with respect to histo plasmos s cocci lio domycos s and blastomycos s and the p oblem of cross re c tions *J Lab & Cl n Med 42* 896 906 19 3

7 Campbell C C Antigenic fractions of histoplasma capsulatum *Am J Pub Health 43* 712 717 1953

8 Cro s F C and Howell A J Studies of fungus antige s II Preliminary repo t on the solation of an immunologically active polysaccharide from histo plasmin *Pub Health Rep 63* 179 183 1948

9 Emmons C W Ol on B J and Eld dge W W Studies on the role of fung in pulmonary d ea e I Cross react ons of hi toplasm n *Pub Health Rep 60* 1383 1394 1945

10 Fur olow M L Bunnell I L and Tenenberg D J A complement f xation te t for histopla mos II Prelim n y esults with human sera *Pub H alth R p 63* 169 172 1948

11 F ircol w M L Emge M F and Bunnell I L Depression of tuberculin and h topla min sens tivity associ ted with critical illness *Pub H alth Rep 63* 1290 1 98 1948

12 G ayst n J T Epidem cs of histoplasmo s In United States Department of Health Education and Welf re *Proc of the Conference n Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 39-45

vidual can stimulate the production of serologically detectable antibodies it appears prudent both to obtain serum for serology prior to repeat skin testing with histoplasmin and to avoid needless skin testing of known skin test positive individuals

SUMMARY AND CONCLUSIONS

(1) *The serology of histoplasmosis has been reviewed with* an attempt made to avoid schematic generalizations Appropriate references for the technics of the various tests are indicated All the serologic tests subjected to clinical trial have proved to be of value

(2) The *precipitin and collodion agglutination tests yield similar results and tend to be positive earlier and for a more tran*sient period than the complement fixation tests This pattern is not constant however and any complement fixation test may be positive in early stages of disease and other tests be negative

(3) The complement fixation test with histoplasmin antigen appears to be the test most likely to be positive in old or inactive appearing disease

(4) The sensitivity of the tests varies not only with the stage of disease represented by the serum but also with different sera from the same stage of disease In addition different lots of an antigen influence the sensitivity and specificity of a given test Therefore the performance of more than one test (e g precipitin or collodion agglutination plus yeast phase and histoplasmin complement fixation) is of more diagnostic value than any single test

(5) A changing titer as well as a significant titer has diagnostic value

(6) Cross reactions occur with other fungus (Blastomyces and Coccidioides) antigens and their antisera Homologous reactions usually can be distinguished from heterologous reactions either by single absolute diagnostic titers or by the method of differential serum titrations When indicated tests with heterologous antigens should be performed

(7) Purified antigenic fractions are not practicable at present

(8) Skin testing as usually performed has no significant

influence upon serologic results Repeated skin testing at short intervals may influence serologic results in skin test positive individuals and should be avoided

(9) There is no reliable prognostic titer or pattern Negative to high titers may be obtained at all stages of infection with each test

(10) Standardized reagents are necessary for valid serologic results Serology is best performed in laboratories experienced with these tests

(11) Isolation of H capsulatum by culture methods is the most valid diagnostic criterion of histoplasmosis

REFERENCES

1 Bo d W C. *Fu damentals of Imm nol gy* Int rscience 1943 p 2 ,

2 Bunnell I L and Furcolow M L A report on ten proved cases of histoplasmosis *Pub Health Rep* 63 299 316 1948

3 Campbell C. C. and Saslaw S Use of yeast phase antigens in a complement fixation test f r h s toplasmosis *Pub Health Rep* 64 551 560 1949

4 Campbell C. C. Use of yea t phase antigens in a complement fixati n test for histopla mosi IV Results with groun l yeast phase antigens in serial specimens of serum from thirty seven patient In United States Department of Health Education and Welfare *Pro of the Conf r nce on Histoplasmos s 195* Pub Health Monogr No 39 19 6 pp 140 143

5 Campbell C C Cross react ons of h ms otic ant gen *Ibid* pp 144 148

6 Campbell C. C. and Binkley C F Serologic diagnosis with respect to h stoplasmos s cocci l oi l myco s s and bla tomycosi an l the prol lem of cros reactions *J Lab & Cl n Med* 4 896 900 1953

7 Campbell C. C Ant genic fractions of histopla ma capsulatum *Am J Pub Health* 43 712 717 19 3

8 Cross F C and Howell A Jr Studies of fungus ant ens II Preliminar repo t on the isolation of an immunolog cally acti e polysaccharide from h sto plasmin *Pub Health Rep* 63 1 9 183 1948

9 Emmons C. W Olson B J and Eldr d e W W Stud es on the role of fungi in pulmonary disease I Cross react ons of h stoplasmin *Pub He lth Pep* 60 1383 1394 194

10 Furcolow M L Bun ell I L nd Tenenberg D J A complement f xation test for hi toplasmos s II P elimina y e ults with human sera *Pub Health Pep* 63 169 1, 1948

11 Furcolow M L Emge M E an l Bunnell I L Depress on of tuberculin and h stoplasmin sensitivity associ ted with critical illness *P b He lth Pep* 63 1 90 1298 1948

12. Grayston J T Ep demics of h stoplasmo s In Unite l States Department of Health Educat n an l Welfare *Proc of the Co ference on H stoplasm sis 1952* Pub Health Monogr No 39 19 6 pp 39-45

13 Grayston J T A study of the complement fixation reaction in histoplasmosis employing whole yeast phase cells as antigen *Ibid* pp 132 139
14 Grayston J T A study of the complement fixation reaction in histoplasmosis *J Lab & Clin Med* 40 90 101 1952
15 Hill G B and Campbell C C A further evaluation of histoplasmin and yeast phase antigen of Histoplasma capsulatum in the complement fixation test *J Lab & Clin Med* 48 2 5 263 195[illegible]
16 Howell A Jr Studies of fungus antigens I Quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs *Pub Health Rep* 62 631 6 1 1947
17 Larsh H W and Furcolow M L Follow up of the Camp Gruber epidemic In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 31 38
18 Lehan P H Brasher C A Larsh H W and Furcolow M L Evaluation of clinical aids to the diagnosis of chronic progressive cavitary histoplasmosis *Am Rev Tuberc* 75 938 948 19[illegible]
19 Martin D S Preliminary experiments on the separation of anti protein and anti carbohydrate antibodies in North American Blastomycosis In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 1956 pp 166 167
20 Norden Ake Agglutination of sheep s erythrocytes sensitized with histoplasmin *Proc Soc Exper Biol & Med* 70 218 220 1949
21 Pates A L Precipitin reactions in experimental histoplasmosis and blastomycosis *Science* 108 383 38 1948
22 Prior J A and Saslaw S Effect of repeated histoplasmin skin tests on skin reactivity and collodion agglutination *Am Rev Tuberc* 66 588 595 1952
23 Rubin H Lehan P H and Furcolow M L Severe non fatal histoplasmosis Report of a typical case with comments on therapy *New England J Med* 57 99 60 1957
24 Sabin A B An epidemic of miliary granulomatous pneumonitis caused by Histoplasma In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 1956 pp 20 23
25 Salvin S B Complement fixation studies in experimental histoplasmosis *Proc Soc Exper Biol & Med* 66 312 315 1947
26 Salvin S B and Hottle G A Serologic studies on antigens from Histoplasma capsulatum Darling *J Immunol* 60 57 C6 1948
27 Salvin S B and Hottle G A Factors influencing histoplasmin formation *J Bact* 56 541 546 1948
28 Salvin S B and Furcolow M L The precipitin test in human histoplasmosis In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 1956 pp 129 131
29 Salvin S B Precipitin and complement fixation tests with whole yeast phase antigen *Ibid* pp 163 165
30 Salvin S B and Furcolow M L Precipitins in human histoplasmosis *J Lab & Clin Med* 43 259 274 1954

31 Salvin S B Weber R W, Lackman D B Nishio J and Menges C Influence of repeated histoplasmin skin tests on precipitins and complement fixing antibodies *J Lab & Clin Med 44* 56 6? 19 4

3? Salvin S B Furc low M L and Nishio J Serologic studies in outbreak of pulmonary disease at Camp Cruber Okla *Arch Int Med* 93 90? 910 19 4

33 Sal in S B and Ribi E Antigens fr m yeast phase of Histoplasma capsulation II Immunologic properties of protoplasm cell walls *Proc Soc Exper Biol & Med* 90 ?8 ?91 19 5

34 Saslaw S and Campbell C C A method for demonstrating antibodies in rabbit sera against histoplasmin by the collodion agglutination technic *Proc Soc Exper Biol & Med* 6? 5 9 ?? 1949

35 Saslaw S and Campbell C C The use of yeast phase antigens in a complement fi ation test for histoplasmosis I Preliminary results with rabbit sera *J Lab & Clin Med* 33 811 818 1948

36 Saslaw S and Campbell C C The use of yeast phase antigens in a complement fixation test for histoplasmosis II Results with ground antigens *Ibid* 33 1?0 1?11 1948

37 Saslaw S and Campbell C C A comparison between histoplasmin and histoplasmin by the collodion agglutination technique *Pub Health Rep* 64 ?90 ?91 1949

38 Saslaw S and Campbell C C A collodion agglutination test for histoplasmosis *Pub Health Rep* 6? 4 4?9 1949

39 Saslaw S The collodion agglutination test In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 13 ? pp ? 3 1 8

40 Saslaw S Collodion agglutination test and complement fixation test with ground yeast phase antigen *Ibid* pp 1 16

41 Saslaw S and Campbell C C Effect of histoplasmin skin testing on serologic results *Proc Soc Exper Biol & Med* 8 ?89 691 19 5

4? Saslaw S and Carlisle H N A histoplasmin latex agglutination test *J Lab & Clin Med* 50 913 19 (Abstract)

43 Schubert J H Evaluation of histoplasmin antigens for the complement fixation test In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 195* Pub Health Monogr No 39 19?6 pp 119 1??

44 Schubert J H Ajello L Stanford S and Grant V Q Variations on complement fixation antigen production by different strains of Histoplasma capsulatum grown on two media *J Lab & Clin Med 41* 91 97 19 3

4 Schubert J H Ajello L Cooper J S and Runyon L C Evaluation of histoplasmin and yeast phase antigens derived from a single strain of Histoplasma capsulatum in the complement fixation test *J Bact 69* 5 8 6? 19

46 Schubert J H and Ajello L Variations in complement fixation antigen city of different yeast phase strains of Histoplasma capsulatum *J Lab & Clin Med* 50 304 307 19

47 Schwarz J and Furcolow M L Some epidemiologic factors and diagnostic tests in blastomycosis coccidioidomycosis and histoplasmosis *Am J Clin Path* 5 ?1 ?6 19

48 Smith C E Saito M T Beard R R Kepp R M Clark R W and Eddie B U Serologic tests in the diagnosis and prognosis of coccidioidomycosis *Am J Hyg* 52 1 21 1950
49 Sorensen L J and Evans E F Antigenic fractions specific for Histoplasma capsulatum in the complement fixation reaction *Proc Soc Exper Biol & Med* 87 339 341 1954
50 Tenenberg D J and Howell Arden Jr A complement fixation test for histoplasmosis I Technic and preliminary results on animal sera *Pub Health Rep* 63 163 168 1948
51 Tenenberg D J Histoplasmin and whole yeast phase Histoplasma capsulatum as antigen in complement fixation tests on histoplasmosis In United States Department of Health Education and Welfare *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 150 152
52 Van Pernis P A Benson M E and Hollinger P H Specific cutaneous reaction with histoplasmosis Preliminary report of another case *J A M A* 117 436-437 1941

THE HISTOPLASMIN SKIN TEST

CARROLL E PALMER AND PHYLLIS Q EDWARDS

The relation of the histoplasmin test to histoplasmosis is as far as is known analogous to the relation of the tuberculin test to tuberculosis and the coccidioidin test to coccidioidomycosis The histoplasmin test however came into use several years later than the coccidioidin test and more than 50 years after the tuberculin test It is therefore not surprising that much of our knowledge and many assumptions about the histoplasmin test are based on earlier experience with other skin tests and particularly on the extensive research studies made on the tuberculin test during the last ten years or so

The rationale of the histoplasmin test is that after infection with H capsulatum the tissues become sensitized to histoplasmin The delayed type of reaction—an indurated erythematous area which reaches its maximum size in two to four days after intracutaneous injection of the antigen—theoretically signifies infection either past or present Absence of such a response theoretically signifies no infection In practice however the histoplasmin test has its limitations as do most biological tests In some instances it is difficult to determine whether or not a reaction is present infection with other fungi may cause reactions which cannot be distinguished from those produced by histoplasmal infection and occasionally the characteristic reaction is absent even though there is evidence that the subject has been infected

Notwithstanding its limitations histoplasmin testing has been a major factor in pointing the way in the accumulation of knowledge about histoplasmosis Results of the first skin testing studies reported in 1945 indicated that sensitivity to histoplasmin was *highly prevalent in some geographic areas and apparently absent* in others (28) and that in areas of high prevalence pulmonary calcification was associated far more often with histoplasmin sensitivity than with tuberculin sensitivity (28 3) From these and later

studies it became evident that histoplasmosis is not a rare and fatal disease. Infection is widespread and usually benign; its prevalence *is largely related to geographic factors. Infection frequently results* in pulmonary calcification, a point of practical importance in clarifying the problem of pulmonary calcification in tuberculin nonreactors—a problem that had obvious implications for the use of the tuberculin test in tuberculosis control work.

Advances in the epidemiology of histoplasmosis may well depend on further developments in histoplasmin testing. One of the problems is to determine how specific reactions can be distinguished from nonspecific or cross reactions caused by infection with other organisms. As the frequency of cross reactions varies with geographic locality, the percentage of reactors to histoplasmin can not always be used as an index of the prevalence of histoplasmal infection.

TECHNIC OF THE HISTOPLASMIN TEST

The histoplasmin test is ordinarily given by injecting 0.1 ml of an appropriate dilution of a standardized antigen into the most superficial layer of the skin of the forearm. If the injection is intracutaneous, as it should be, a small bleb is raised in the skin. As the size of the bleb will vary with the depth of injection (29), the 0.1 ml volume should be measured as exactly as possible by using the fine calibrations on the barrel of a 1 ml syringe. The histoplasmin test is a quantitative, not a qualitative test, and it is perhaps fortunate that few serious attempts have been made to "simplify" it by substituting for the intracutaneous procedure an even less precise qualitative procedure, such as some form of a patch test.

Histoplasmin, like tuberculin and coccidioidin, may adhere to or be adsorbed onto the surfaces of needles, syringes and glassware or containers used in preparing or storing the antigen. Chemically clean glassware or, preferably, all new glassware should there*fore be used to* avoid possible contamination of the histoplasmin by other antigens.

In unsensitized persons the histoplasmin test generally evokes no reaction; the only sign that a test has been given is a tiny mark caused by the trauma of the needle. In the sensitized person an

area of infiltration (induration) usually surrounded by an erythematous flush begins to appear at the site of injection within 24 hours reaches its maximum size between the second and fourth day and then gradually disappears Reading of the reaction is therefore made at either 48 or 72 hours after the injection The area of induration should be palpated carefully and its widest transverse diameter measured with a millimeter ruler The measurement is recorded for as is shown later the size of the reaction is important in its interpretation Erythema without palpable induration may also be measured and recorded but its significance as an index of histoplasmal infection has not been carefully studied

The histoplasmin reaction is indistinguishable in appearance from the reaction to tuberculin or to coccidioidin Very large reactions bullae necrosis or lymphangitis occasionally seen with tuberculin reactions are much less frequent with the dilution of histoplasmin ordinarily recommended for skin testing

THE SKIN TEST ANTIGEN HISTOPLASMIN

The first report of a skin test for histoplasmosis was published in 1941 by Van Pernis *et al* (39) who used an antigen prepared from a culture filtrate of H capsulatum grown in dextrose broth Zarafonetis and Lindberg also in 1941 reported the preparation of an antigen from a culture filtrate and proposed that the antigen be called histoplasmin (43) In 1945 Emmons *et al* of the Public Health Service described the preparation of several lots of histoplasmin

The histoplasmin used in our investigations was prepared by growing strains of H capsulatum on a synthetic broth medium made according to the formula recommended by Dr C E Smith (36) for the preparation of coccidioidin

l Asparagin	14 00 gm
Dipotassium phosphate c p (K_2HPO_4)	1 31 gm
Sodium citrate c p ($Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$)	0 90 gm
Magnesium sulphate (U S P) ($MgSO_4 \cdot 7H_2O$)	1.50 gm
Ferric citrate (U.S P) VIII (Scales)	0.30 gm
Dextrose of the grade known as Cerelose (U.S P X)	10 00 gm
Glycerine c p (U.S P)	25 00 gm
Water to make	1,000 00 ml

This is similar to the medium used in making tuberculin Experience has shown that this culture medium itself does not sensitize or elicit nonspecific reactions The medium was dis pensed in 3 liter Erlenmeyer flasks 1500 ml per flask and auto claved Bits of dry mycelium from agar slant cultures of H cap sulatum were floated on the surface of the broth and the inocu lated cultures were then incubated in a dark cupboard at room temperature for periods varying from 2 to 4 months (in one lot 7 months) At the end of the incubation period the flask was shaken to immerse all the floating mycelium and on the follow ing day the broth was filtered through a Berkefeld N filter and tested for sterility Merthiolate was added to give a final concen tration of 1 10 000 and the material was bottled without either concentration or restoration to original volume The color of the filtrate was a clear amber (12)

Results with the lot identified as H 3 prepared according to these specifications were reported by Emmons *et al* from studies on infected and uninfected guinea pigs and also on hospitalized mental patients (12) On Emmons recommendation Palmer used the 1 1000 dilution of H 3 for testing student nurses in various parts of the country (28) On empirical grounds the 1 1000 dilu tion appeared to be satisfactory because a high percentage of reac tors was found in some areas and a negligible percentage in others Christie and Peterson at Vanderbilt University also reported satisfactory results from testing children in Tennessee with a 1 100 dilution of the histoplasmin they had prepared from a broth culture filtrate (3) Subsequently a number of other workers both in the United States and other countries have prepared histoplasmin but the products most widely used have been the series designated as H 15 H 10 and H 42 prepared by Howell of the Public Health Service (35) His method of preparation was very similar to that of Emmons and each successive lot was standardized and the potency adjusted so the dilution used for testing was as nearly as possible the same as the 1 1000 dilution of Emmons H 3

Histoplasmin H 42 prepared in 1947 was made by pooling three separate lots in order to obtain one product in sufficient quantity to last for a number of years (35) An estimated several hundred thousand tests have already been given in this and other

countries with H-42 and several million more tests can be given with the amount still available (30) H-42 has been used since 1952 by the Biologics Control Laboratory of the National Institutes of Health as Reference Histoplasmin No 1 for the standardization of products from other sources (42)

Standardization

The Laboratory of Biologics Control prepared minimum requirements for the production of histoplasmin in 1952 (27) According to Workman and Hottle these requirements should

> ensure that every laboratory which plans to distribute histo plasmin not only is capable of producing a product which is acceptable to the clinician from the viewpoint of purity and safety but also continues to produce a product of uniform potency (42)

The requirements specify that either human subjects or sensitized guinea pigs may be used for potency tests carried out by means of intracutaneous tests with serial two fold dilutions of both the reference product supplied by the National Institutes of Health and the product under test If guinea pigs are used four adequately sensitized animals are considered necessary for a satisfactory test (42)

Stability

Undiluted stock histoplasmin stored in the refrigerator (at about 5 C) does not appear to lose its potency over a period of years (19 30) There is evidence however that dilutions are less stable than the undiluted antigen particularly if subjected to light or to temperatures above refrigeration For that reason use of diluted histoplasmin for more than eight weeks is generally not recommended

Dosage

The dose of histoplasmin for skin testing is expressed in terms of the dilution of the particular product which in a volume of 0 1 ml has a potency adjusted to match the potency of 0 1 ml of the Reference Histoplasmin For some products this may mean that the original histoplasmin must be diluted by 1 100 or 1 1000 or some other ratio to give the appropriate dosage for the skin test in 0 1 ml For H-42 for example the concentration of the original

histoplasmin was adjusted so that a 1 100 dilution of the adjusted stock material contains the appropriate dosage

Although the 1 100 dilution of H-42 (or other histoplasmins adjusted to have an equivalent potency) is currently accepted as satisfactory for skin testing of human populations it should be realized that this dose was arrived at empirically Recent studies suggest that more precise results might be obtained with a slightly stronger concentration (30) Basically however the question of the potency of a skin test antigen can not be considered apart from the question of its specificity

Specificity

The specificity of histoplasmin has been studied in laboratory animals experimentally infected with the homologous and several heterologous fungi including Blastomyces dermatitidis Coccidioides immitis and Haplosporangium parvum Emmons *et al* (12) reported cross reactions between all four of the corresponding antigens and a particularly high degree of cross between histoplasmin and blastomycin Subsequent investigations by Howell (20) focused attention on the relation between what he called the critical titer of the antigen and the frequency of cross reactions showing that the frequency and size of cross reactions are related to the dosage the particular antigen used for testing and the degree of sensitivity of the test animals

Attempts to isolate the active principle from histoplasmin have been reported by Cross and Howell (4) who found that a polysaccharide fraction free of protein gave reactions in guinea pigs experimentally infected with either Histoplasma or Blastomyces When used in appropriate doses the polysaccharide antigen was relatively specific for histoplasmal infection Dyson and Evans subsequently reported from their experimental studies of histoplasmin prepared from the yeast phase of the fungus that Although the skin test antigen was originally thought to be polysaccharide the extraction of aqueous solutions with chloroform in order to remove protein was also found to destroy the reactivity of the antigen The polysaccharide which remained following this treatment appeared to be inert in the delayed skin reactions This points up the need for additional chemical studies which are now in prog

ress (5) More recently however Knight and Marcus (21) have been able to extract specific soluble polysaccharides from yeast phase broth cultures of H capsulatum

Results of field studies in human beings (11 31 38 14) have paralleled those from the laboratory although in field work it is rarely possible to know as it is in the laboratory whether an individual or a group of individuals has been infected with one particular organism with several different organisms or with none at all Histoplasmin appears to be a much less specific antigen than for example coccidioidin that is the histoplasmin test elicits a response not only in persons infected with Histoplasma but in a substantial proportion (in the neighborhood of 40%) of those infected with Coccidioides as well Coccidioidin in contrast elicits reactions in only about 2% of the histoplasmal infected There is ample evidence that histoplasmin and tuberculin do not cross react The latter part of this chapter will deal in greater detail with the characteristics of specific (homologous) and nonspecific (heterologous or cross) reactions to histoplasmin based on results of field studies in human populations

GENERAL CHARACTERISTICS OF HISTOPLASMIN SENSITIVITY

When the histoplasmin test first came into general use reactions were read as positive or negative A positive was defined as a reaction of 5 or more mm of induration taken to indicate infection by Histoplasma a negative as less than 5 mm indicating no infection That criterion was being used at the time to classify tuberculin and coccidioidin reactions and was simply taken over quite arbitrarily for classifying histoplasmin reactions Although we now know that the 5 mm criterion is often inapplicable it unquestionably served a very useful purpose in establishing certain basic characteristics of histoplasmin sensitivity and histoplasmal infection which pointed the way for many of the later contributions from laboratory clinical and pathological studies

Time of Appearance of Sensitivity

In experimental animals skin sensitivity to histoplasmin apparently develops within a few weeks after infection with H capsulatum (20) In human beings the time interval between infection and appearance of skin sensitivity is also probably a few weeks as would be expected if histoplasmosis is analogous to tuberculosis in this respect Loosli s report of an epidemic in an Indiana farm family indicates that the histoplasmin reaction had developed by the eighth week after exposure to the source of infection (23) Observations on a small group of persons who developed clinical histoplasmosis shortly after visiting a bat infested cave near the village of Sarare in the State of Lara Venezuela reported by Campins and his associates (2) showed histoplasmin sensitivity to be present by the end of the fourth week after exposure Similar time intervals have been reported by Murray *et al* (26) in the Transvaal although Sabin s observations on a group of men involved in a localized epidemic in Cincinnati (33) suggest that the time of appearance of sensitivity may be quite variable

Duration of Sensitivity

The duration of skin sensitivity to histoplasmin seems to be many years perhaps lifelong according to the impression of many workers Smith for example cites six persons from the midwest who after living for 10 to 20 years in California still retain their histoplasmin sensitivity presumably in the absence of exposure to Histoplasma during that interval (37) Furcolow and Grayston made a follow up study in 1953 of 94 out of 116 persons involved in localized epidemics of histoplasmosis as long as nine years previously and reported that in all 94 instances sensitivity to histoplasmin was present at the time of the follow up (15) In contrast Zeidberg has reported a sharp rise in the prevalence of reactors among children but a gradually declining prevalence with age in the adult population of an endemic area he conjectures that the lower prevalence among adults may mean loss of acquired sensitivity less exposure to the sensitizing agent or the acquisition of immunity (44) So far as is known at the present time however no systematic long term studies have yet been made that would provide

firm data on the question of the duration of histoplasmin sensitivity

Suppression of Sensitivity

Sensitivity may be suppressed during an acute illness as well as in the terminal stages of an illness As shown by Furcolow and his associates some years ago (13) from studies of 305 patients on the critical list at a municipal hospital in Kansas City depression of skin sensitivity appears to be a function of the critical illness itself—it is not specific to a particular disease—and occurs with both tuberculin and histoplasmin sensitivity Age also seems to be a factor as depression of sensitivity was found to increase in frequency with increasing age of the critically ill patient

Degree of Sensitivity and Infecting Dose

A relation between the degree of sensitivity and the dose of infecting organisms has not been demonstrated in human beings and would on the face of it be an extremely difficult relation to study directly Results of extensive research on tuberculin sensitivity has indicated that the degree of sensitivity exhibited by an individual is determined largely by familial factors governing his capacity to develop sensitivity (32) neither the size of the infecting dose nor the frequency of repeated infections appears to have much influence on the degree of sensitivity as estimated by the size of the skin reaction in humans (10 6) There are some indications that here again the analogy may hold between tuberculin and histoplasmin sensitivity (31 8)

FACTORS AFFECTING ACQUISITION OF SENSITIVITY

Place of Residence

The prevalence of sensitivity to histoplasmin in human populations varies widely with geographic locality As shown by the map in Figure 1 based on results of skin testing surveys reported in the literature up to 1955 (10) histoplasmin sensitivity is highly prevalent in parts of the Americas with scattered low prevalence areas in some regions of Africa and Southeastern Asia Few if any reactors have been found in Europe the eastern Mediterranean or the Asiatic countries from which studies have been reported

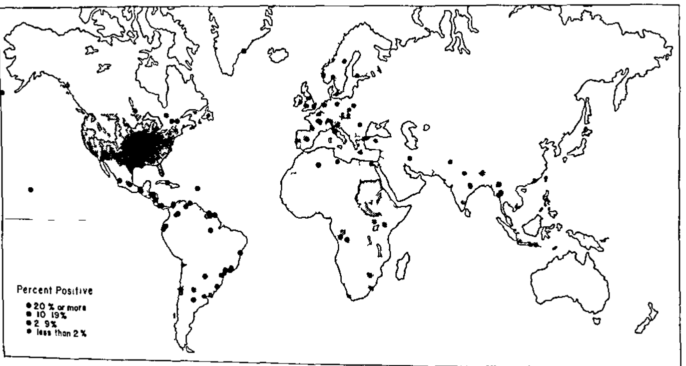


Fig 1 Percentages of histoplasmin reactions reported as positive from surveys conducted in various parts of the world

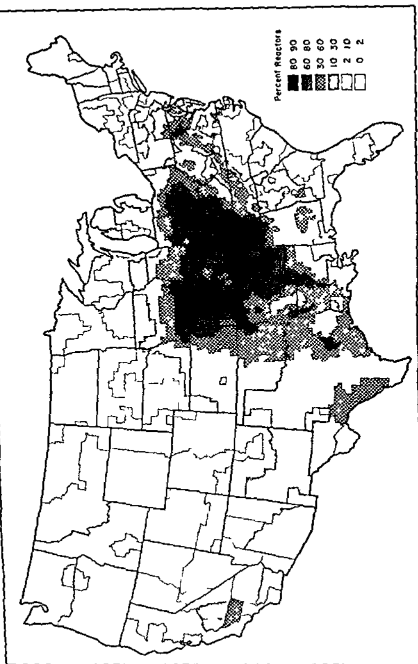


Fig 2 Percentag s of histoplasmin reactions (5 or more millimeters) among young adults lifetime residents of single counties of the United States

A more detailed map of the geographic distribution of histoplasmin sensitivity in the United States is presented in Figure 2. The map shows the distribution of counties and groups of counties according to the prevalence of reactions to histoplasmin measuring 5 or more mm among a total of 68 152 young adult men and women tested between the years 1945 and 1951 in studies carried out by the Public Health Service (25). Only lifetime residents of single counties were included in the material to give a reasonably accurate picture of geographic variations in the prevalence of histoplasmin sensitivity. The area of highest prevalence lies in the Eastcentral states where the frequencies of reactors in some localities exceed 80%. The frequencies decrease with increasing distance from this endemic focus. Histoplasmin sensitivity is essentially absent in lifetime residents of New England, the South Atlantic and entire Northwestern regions of the country.

When the map in Figure 2 was prepared, the criterion of 5 mm was used in estimating the frequency of positive reactions although it was recognized that a fair proportion of the positive reactions found in the Southwestern states probably represented nonspecific or cross reactions caused by Coccidioides infection. More recent studies (11, 31, 8) have confirmed that interpretation and have, in addition, shown that many of the reactions in the region centering in eastern Texas are also nonspecific, although they cannot be ascribed to coccidioidal infection.

Numerous reports have been made on geographic variations in the prevalence of histoplasmin sensitivity within single counties (7, 9, 17, 34). In Jackson County, Iowa, for example, the percentage of reactors ranged from 7 to 28 among school children living in the 18 townships of the county (17). Differences of similar magnitude were reported from Montgomery County, Maryland, where within a distance of no more than 20 miles the reactor rate among high school students jumped from about 14% to more than 60% (7). Even sharper differences were observed in studies of school children in the city of Dalton, Georgia (1). Within a radius of only 3 miles children who lived in one particular section of the city showed a frequency of reactors twice as high as those who lived in other sections. Such abrupt changes in reactor rates over very short distances

seem to point to highly concentrated sources of infection Whatever the mechanism of transmission infection would seem to depend on direct exposure to the place where the fungus exists (2 15 23 26 41)

Other Factors

Race sex occupation socio economic status rural or urban residence and other factors may influence the prevalence of histo plasmin sensitivity but it is difficult to isolate their effect from the over riding effect of geographic factors associated with place of residence The frequency of reactors tends to be slightly higher in males than in females after the age of about 12 years it is often somewhat higher in whites than in Negroes in this country and it is generally higher in farm than in urban residents However in the study of Georgia school children referred to above (1) a much higher frequency was found in children living in a well to-do sec tion of the city of Dalton than in the surrounding rural areas

Although concentration of histoplasmin reactors in some fami lies and not in others has been observed repeatedly the possibility of familial susceptibility or resistance to infection obviously is dif ficult to separate from the strong influence of a common environ ment on family groups

Manos has summarized a number of studies of the relation between age and the prevalence of histoplasmin reactors (24) Regardless of the prevalence of sensitivity in a community appar ently very few children become sensitive in the first few years of life From the second or third year of age there is a progressive increase in the frequency of reactors up until adulthood but the *rate* of increase varies with the geographic area In some areas of course the rate may be very low or zero while in high endemic areas the annual rate with which nonreactors become reactors to histo plasmin may be as high as 10 per cent

INTERPRETATION OF REACTIONS TO HISTOPLASMIN

Theoretically a histoplasmin test might be expected to pro duce definite clearly characteristic reactions in persons who have been infected by Histoplasma and no reaction in persons who have

not been infected In practice however errors in giving the test variations in the size of reactions and other factors make it difficult in some instances to judge whether an individual reaction should be interpreted to mean infection or no infection The commonly used definition that a reaction of 5 or more mm in diameter indicates infection and one measuring less than 5 mm indicates no infection should be regarded as only a crude and sometimes quite inadequate criterion

The problem is illustrated in Figures 3 4 and 5 Figure 3 shows the results of testing 1018 high school students in Whitfield and Murray Counties Georgia with the 1 100 dilution of histoplasmin H-42 (9) The size of the reaction is indicated by the horizontal scale and the percentage of the total group with reactions of specified size is indicated by the height of each column At first glance it can be seen that the frequency distribution has two peaks one at 0 1 mm and the other at 12 13 mm From the form of the

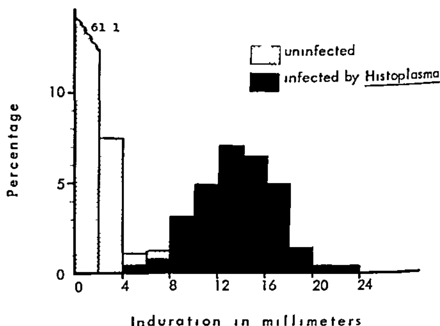


Fig 3 Frequency distribution of sizes of reactions to histoplasmin for 1 018 high school students in Whitfield and Murray Counties Georgia

distribution it appears that according to their size the reactions fall into two groups which overlap in the range between 4 and 8 mm Reactions of 2 and 3 mm would seem to belong with the left hand group with those of 0 and 1 mm and reactions of 8 or more mm with the right hand group Reactions of 4 to 8 mm appear to represent a mixture with most of the 4 and 5 mm reactions probably belonging to the left hand group and most of the 6 and 7 mm reactions probably belonging to the right hand group The point between 5 and 6 mm thus appears to be about the best place to separate the total distribution into two groups Thus the 1 100 dilution of H-42 appears to discriminate with some precision this particular population into two groups Nevertheless it should be emphasized that individual reactions of 5 and 6 mm and some of those measuring a few millimeters larger or smaller cannot be allocated with much precision to either group

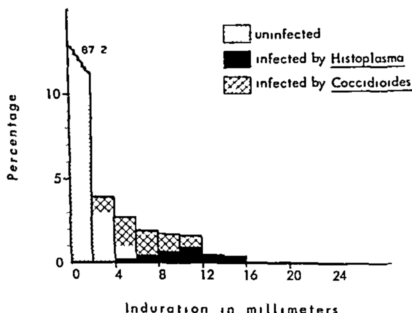


Fig 4 Frequency distribution of sizes of reactions to histoplasmin for 4 521 Navy recruits lifetime residents of southern California Arizona New Mexico and western Texas

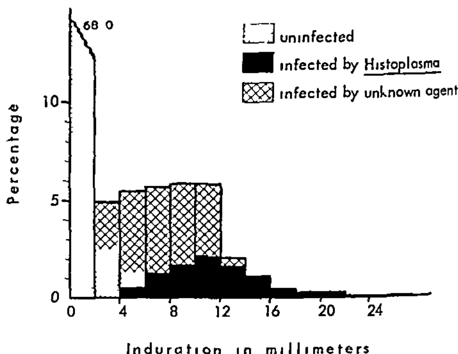


Fig 5 Frequency distribution of sizes of reactions to histoplasmin for 3 636 Navy recruits lifetime residents of western Kansas and Oklahoma eastern Texas and western Louisiana

The next question is whether or not the two groups discriminated by the test correspond to the infected and uninfected segments of the population For this purpose some criterion of infection must be used which is independent of the skin test itself While serological and mycological methods are available as diagnostic tests in persons recently infected or with clinically active disease one of the few signs of past infection with Histoplasma in a healthy person is x ray evidence of pulmonary calcification (or infiltration) Thus if other causes of pulmonary calcification notably tuberculosis and coccidioidomycosis can be identified and excluded then the relation between calcification and size of reaction to histoplasmin can give a rough indication of whether or not the two groups distinguished by the skin test correspond to the infected and uninfected segments of the population

The results of such an analysis for the students of Whitfield-Murray Counties is presented in Table I. The 70 mm photofluorograms taken of all students were read independently by two chest physicians without knowledge of the skin test results. Films interpreted as showing either calcification or infiltration by both readers were, as shown in the table, highly concentrated in the group with reactions to histoplasmin measuring at least 6 mm: 44 out of 307 (14%) had pulmonary findings. One of the 44 also reacted to tuberculin and none reacted to coccidioidin. In contrast only 2 out of 711 students (0.3%) with small or zero reactions had pulmonary findings. The x-ray findings thus support interpretation of the two groups distinguished by the histoplasmin test as corresponding to the infected and uninfected segments of the study population.

Unfortunately, the interpretation of histoplasmin reactions is sometimes much less clear-cut than it appears to be for the students

TABLE I

RELATION BETWEEN SIZE OF REACTION TO HISTOPLASMIN AND FREQUENCY OF PULMONARY CALCIFICATIONS OR INFILTRATIONS AMONG HIGH SCHOOL STUDENTS, WHITFIELD-MURRAY COUNTIES, GEORGIA

Size of Reaction to Histoplasmin (in millimeters)	*Number of Students Tested*	*Number with Pulmonary Findings*
0–1	693	2
2–3	6	—
4–5	12	—
6–7	13	
8–9	33	3
10–11	50	3
12–13	72	15
14–15	66	10
16–17	51	8
18–19	14	2
20–21	4	1
22–23	4	—
Total	1,018	46

Includes one person with a reaction of 18 mm to the 5 TU tuberculin test.

in Whitfield Murray Counties The frequency histogram presented in Figure 4 illustrates one type of complication that is when some of the population being tested have been infected with Coccidioides immitis The material for this graph was obtained from tests given to a group of young men Navy recruits as they entered the Navy Training Center at San Diego California (8 11 25 31) Results are included in the figure only for recruits who had lived all their lives in the endemic coccidioidomycosis region in the Southwest—southern California Arizona New Mexico and western Texas

The form of the distribution of reactions in Figure 4 differs strikingly from that seen in the previous figure A relative increase in the proportion of small reactions has completely obliterated the point at which two groups could be separated In fact as shown by the shading of the columns we are dealing here not with two groups but with three 1) those not infected by either Histoplasma or Coccidioides 2) those infected by Histoplasma but not by Coccidioides and 3) those presumably infected by Coccidioides but not by Histoplasma

The first group have reactions similar to the uninfected group in Whitfield Murray Counties most of them have no reaction or a very small one They are classified as uninfected In the second and relatively small group the distribution of reactions according to their size is similar to that of the infected group in Whitfield Murray Counties their reactions are attributed to histoplasmal infection Finally the persons in the third group had larger reactions to coccidioidin than to histoplasmin which is one indication that they may have had a coccidioidal rather than histoplasmal infection but more importantly their reactions to histoplasmin are generally smaller than the reactions of persons living in the Histoplasma endemic area These small histoplasmin reactions are interpreted as cross reactions

Figure 5 shows the distribution of histoplasmin reactions in Navy recruits from another part of the country—western Kansas and Oklahoma eastern Texas and western Louisiana Here again the distribution is interpreted as representing three different kinds of reactions 1) negatives 2) specific reactions caused by histoplasmal

infections and 3) cross reactions The cross reactions although similar in size to those attributed to coccidioidal infection in the southwest can not be caused by Coccidioides in this region as less than 2% of the total group of recruits reacted to coccodioidin Moreover these reactions were found to be associated with a very low frequency of pulmonary calcification They must represent infection with some other organism most likely a fungus which does not produce pulmonary calcification and whose identity is not known at the present time

The foregoing material illustrates how the classification of histoplasmin reactions according to an arbitrary criterion such as 5 mm or more for a positive reaction and less than 5 mm for a negative reaction may be quite satisfactory in some situations but quite misleading in others Place of residence can not be disregarded as it has an important influence on the frequency of infections that cause cross reactions with histoplasmin As a consequence it is a mistake to expect that a simple rule can be laid down which will apply equally well for the interpretation of histoplasmin reactions where ever they are found

The interpretation of histoplasmin reactions for groups of persons must take into consideration the results of the study of the frequency distribution of the sizes of the reactions carefully measured of a representative sample of the residents of the locality For individuals account must also be taken of past history of residence Histoplasmin reactions in persons who have lived in the southwest for example may be cross reactions caused by coccidioidal infection If the histoplasmin reaction is large say 12 or more mm it is not likely to be a cross reaction but the smaller the histoplasmin reaction the more likely it is to be a cross reaction and testing also with coccidioidin becomes increasingly more important to clarify the sourre of the sensitivity As knowledge of different sources of cross reactions increases it seems inevitable that a battery of antigens will have to be used unless of course specific antigens become available and the problem of cross reactions then becomes only of historical interest

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18 High R H and Zwerling H B Variation with age in the frequency of tuberculous pulmonary calcification *Pub Health Rep* *61* 1,69 178? 1946
19 Hillegas A B Availability of standardized histoplasmin Proc Conf Histoplasmosis pp 22, 231 Pub Health Serv Pub No 46 U S Govt Printing Off Washington D C 1956
20 Howell A Jr Studies of fungus antigens quantitative studies of cross reactions between histoplasmin and blastomycin in guinea pigs *Pub Health Rep* 6 631-6,1 1947
21 Knight R A., and Marcus S Polysaccharide skin test antigens derived from Histoplasma capsulatum and Blastomyces dermatitidis *Am Rev Tuberc* *77* 983 989 19,8
22 Loosli C C Beadenkopf W G Rice F A and Savage L J Epidemiological aspects of histoplasmin tuberculin and coccidioidin sensitivity *Am J Hyg* *53* 33 57 19 1
23 Loosli C G Grayston J T Alexander E R and Tanz F Epidemiological studies of pulmonary histoplasmosis in a farm family *Am J Hyg* *55* 39 401 19,2
24 Manos N E Histoplasmin sensitivity conversion rates *Am J Hyg* 58 119 131 19,3
25 Manos N E, Ferebee S H and Kerschbaum W F Geographic variation in the prevalence of histoplasmin sensitivity *Dis Chest* 9 619 668 19 6
26 Murray J F Lurie H I Kaye J Komins C Borok R and Way M Benign pulmonary histoplasmosis (cave disease) in South Africa *South African M J* *31* 15 2,3 19 7
27 National Institutes of Health Minimum Requirements Histoplasmin Bethesda Md The Institutes 19,
28 Palmer C E Nontuberculous pulmonary calcification and sensitivity to histoplasmin *Pub Health Rep* *60* 513 520 1945
29 Palmer C E and Edwards P Q Variation in technique of intracutaneous BCG vaccination *B it M J* *1* 363 378 19 3
30 Palmer C E and Edwards P Q The dose of histoplasmin H-4 for skin testing *Am Rev Tuberc* *77* 546 550 1958
31 Palmer C E Edwards P Q and Allfather W E Characteristics of skin reactions to coccidioidin and histoplasmin with evidence of an unidentified source of sensitization *Am J Hyg* *66* 196 213 19 7
32 Palmer C E and Nyboe Meyer S Research contributions of BCG vaccination programs I Tuberculin allergy as a family trait *Pub Health Rep* *66* 59 ?,6 1951
33 Sabin A B An epidemic of miliary granulomatous pneumonitis caused by histoplasma Proc Conf Histoplasmosis pp 20 3 Pub Health Serv Pub No 46 U S Govt Printing Off Washington D C 19,6
34 Sachs D Smith R T Fleming D S and Furcolow M L The prevalence of positive reactions to tuberculin and histoplasmin in rural Minnesota county *Am J Hyg* *6* 43 53 19 5
35 Shaw L W Howell A Jr and Weiss E S Biological assay of lots of histoplasmin and the selection of a new working lot *Pub Health Rep* *65* 583 609 1950
36 Smith C E Coccidioidomycosis *M Cl North America* 790 807 1943

REFERENCES

1 Aronson D L and Edwards P Q An urban focus of histoplasmin sensitivity *Am Rev Tuberc 79* 83 86 1959

2 Campins H Zubillaga C Z Lopez L G and Dorante M An epidemic of histoplasmosis in Venezuela *Am J Trop Med 5* 690 695 1956

3 Christie A and Peterson J C Pulmonary calcification in negative reactors to tuberculin *Am J Pub Health 35* 1131 1147 1945

4 Cross F W and Howell A Jr Studies on fungus antigens Preliminary report on the isolation of an immunologically active polysaccharide from histoplasmin *Pub Health Rep 63* 179 183 1948

5 Dyson J E and Evans E E Delayed hypersensitivity in experimental fungus infections skin reactivity of antigens from the yeast phase of Histoplasma capsulatum *J Lab & Clin Med 45* 449 454 1955

6 Edwards L B and Palmer C E Epidemiological studies of tuberculin sensitivity I Preliminary results with purified protein derivatives prepared from atypical acid fast organisms *Am J Hyg 68* 213 231 1957

7 Edwards L B Peeples W J and Berger A G Prevalence of sensitivity to tuberculin and histoplasmin among high school students in Montgomery County Maryland *Pediatrics 21* 389 396 1958

8 Edwards P Q Histoplasmin testing in different geographic areas *Lancet ii* 707 712 1957

9 Edwards P Q Jacobs C F and Barfield D Sensitivity to tuberculin histoplasmin and coccidioidin among high school students in Northwestern Georgia *Dis Chest 34* 167 483 19 8

10 Edwards P Q and Klaer J H World wide geographic distribution of histoplasmosis and histoplasmin sensitivity *Am J Trop Med 5* 235 257 1956

11 Edwards P Q and Palmer C E Prevalence of sensitivity to coccidioidin with special reference to specific and nonspecific reactions to coccidioidin and to histoplasmin *Dis Chest 31* 35 60 1957

12 Emmons C W Olson B J and Eldridge W W Studies of the role of fungi in pulmonary disease cross reactions of histoplasmin *Pub Health Rep 60* 1383 1394 1945

13 Furcolow M L Emge M E and Bunnell I L Depression of tuberculin and histoplasmin sensitivity associated with critical illness *Pub Health Rep 63* 1290 1298 1948

14 Furcolow M L Federspiel C F and Larsh H W Histoplasmin coccidioidin and tuberculin sensivity among school children in two Texas counties *Pub Health Rep 70* 12 18 1955

15 Furcolow M L and Grayston J T Occurrence of histoplasmosis in epidemics etiologic studies *Am Rev Tuberc 68* 307 320 1953

16 Furcolow M L Schwarz J Hewell B A and Grayston J T Incidence of tuberculin histoplasmin and blastomycin reactors among a group of school children *Am J Pub Health 43* 1523 1531 1953

17 Grayston J T Furcolow M L and Heeren R Geographic distribution of histoplasmin reactors among school age children in Jackson County Iowa Proc. Conf Histoplasmosis pp 201 209 Pub Health Serv Pub No 465 U S Govt Printing Off Washington D C 1956

THE IDENTIFICATION OF HISTOPLASMA IN SMEARS AND MICROSECTIONS BY STAINING METHODS*

ROBERT W. MOWRY AND J. K. FRENKEL

I. RECENT PROGRESS IN THE DEMONSTRATION OF HISTOPLASMA AND OTHER FUNGI IN PATHOLOGIC TISSUES

It is a credit to earlier workers that so many cases were diagnosed as histoplasmosis by mere recognition of the organisms in paraffin sections stained only by hematoxylin and eosin. We now have better methods. The great promise of modern histochemical procedures for the study of fungi in pathologic tissues was first suggested in 1947 by Lillie (20). In this communication he described the greatly enhanced demonstration of fungi afforded in paraffin sections by application of the Bauer reaction (2). This consisted of chromic acid oxidation followed by exposure to Schiff's leucofuchsin reagent. The Bauer reaction is now believed to depend on the oxidation to aldehydes of vicinal hydroxyl groups plentiful in most but not all polysaccharides. The following is a quotation describing the results when the Bauer reaction was applied to paraffin sections containing Histoplasma. "Organisms were readily identified as fine red purplish rings surrounding a clear zone about the centrally placed blue or pale blue nucleus. (Authors' note: any *blue* coloration of nuclei would be due to the hematoxylin counterstain.) After salivary digestion the Histoplasma capsules were still Bauer positive. Ptyalin and diastase tests gave the same results in the five animals so tested." From these observations Lillie had shown that the cell wall or so-called capsule contained poly

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37 Smith C F Analogy of coccidioidin and histoplasmin sensitivity Proc Conf Histoplasmosis pp 173 177 Pub Health Serv Pub No 465 U S Govt Printing Off Washington D C 1956
38 Smith C E Saito M T Beard R R Rosenberger H G and Whiting E G Histoplasmin sensitivity and coccidioidal infection occurrence of cross reactions *Am J Pub Health 39* (2) 736 1949
39 Van Pernis P A Benson M F and Holinger P H Specific cutaneous reactions with histoplasmosis Preliminary report of another case *J A M A 117* 436 437 1941
40 WHO Tuberculosis Research Office Further Studies of Geographic Variation in Naturally Acquired Tuberculin Sensitivity *Bull World Health Organ 12* 63 83 1955
41 Wilcox K R Waisbren B A and Martin J The Walworth Wisconsin epidemic of histoplasmosis *Ann Int Med 49* 388 418 1958
42 Workman W G and Hottle G A Standardization of histoplasmin Proc Conf Histoplasmosis pp 221 224 Pub Health Serv Pub No 465 U S Govt Printing Off Washington D C 1956
43 Zarafonetis C J D and Lindberg R B Histoplasmosis of Darling Observations on the antigenic properties of the causative agent A preliminary report *Univ Hosp Bull Ann Arbor 7* 47-48 1941
44 Zeidberg L D The microdistribution of histoplasmin sensitivity in an endemic area Proc Conf Histoplasmosis pp 190 197 Pub Health Serv Pub No 465 U S Govt Printing Off Washington D C 1956

negligible staining of other tissue constituents produced sharper contrast of fungi against tissue Gridley (1952) combined the Bauer stain with Gomori's aldehyde fuchsin an empirical stain found to color hyphae of certain fungi not well shown by the PAS (12) A yellow counterstain was used to heighten the contrast of organisms with the background The various oxidant Schiff methods produced such improved results that Puckett (39) and Zimmerman (16) were able to show that many so-called tuberculomas of the lung were actually instances of old presumably inactive histoplasmosis In cases previously suspected as tuberculous many such lesions contained structures consistent in appearance with Histoplasma when none were visible with routine stains and cultures failed

Histoplasma are not as easily detected in pathologic tissues by the PAS or the Gridley stain as the other yeast like fungi In cases of histoplasmosis the organisms are often colored but moderately by the usual PAS and the pathologist may have difficulty in the interpretation of such sections For this reason many pathologists now prefer a procedure devised by Gomori (1946) (9) and applied to fungi by Grocott (1955) (13) This consists of chromic acid oxidation followed by methenamine silver instead of Schiff's leuco fuchsin and is derived from the Bauer reaction It has been known that the oxidation products from some polysaccharides—though not all—can be colored by alkaline silver nitrate instead of Schiff's reagent Happily methenamine silver after chromic acid produced stronger coloration of Histoplasma than usually seen after oxidant Schiff methods while at the same time producing less coloration of the tissues generally This resulted in greatly improved contrast in most instances Grocott's method is now considered by many workers to be the method of choice for the identification of Histoplasma (1 43 44 47)

Potenza and Feo (1956) called attention to the fact that fungi were often birefringent in paraffin sections both unstained and after the H&E and PAS stains (38) While the presence of fungi in tissues is occasionally revealed in brilliant fashion by microscopic study under polarized light experience with this method is limited We doubt that it will prove useful as a general means of detecting fungi in pathologic tissues Very recent work by Sweany (43 44) and his

saccharide stainable by the aldehyde that resulted from oxidation with chromic acid Lillie also showed that dead Histoplasma—lacking nuclei—could still be identified by staining the carbohydrate rich cell wall In another subsequently studied animal numerous Bauer positive rings without central nuclei were found in the foam cell granulation tissue where no Histoplasma could be found with azure eosin stains This indicates that this method may be used to demonstrate non nucleated and presumably dead Histoplasma as well as the nucleated and presumably surviving forms Important observations on other pathogenic fungi were also included in this paper that is replete with details concerning fungi protozoa as well as bacteria and even the staining of metazoa

Beginning in 1950 a series of papers by Kligman and co workers from the Department of Dermatology at the University of Pennsylvania established the decisive value of the recently introduced periodic acid Schiff (PAS) reaction for the coloration of fungi in smears and tissue sections (17 19) They believed that the PAS method was superior to all preexisting ones and should supplant the Bauer reaction These workers recorded and illustrated the morphologic features revealed by the PAS reaction with the various common pathogenic fungi in both human and experimental pathologic lesions Tissue forms of most fungi were deeply colored But Histoplasma were not colored so deeply as the other pathogenic yeasts Also Actinomyces and certain hyphae were poorly colored In Histoplasma the nuclei were often colored by the PAS reaction but *the stronger and more consistent reaction was in the cell wall* Budding forms of Histoplasma were more easily found in sections stained by the PAS *More drastic conditions of periodic oxidation produced stronger coloration of cell walls of Histoplasma* but weaker or negligible coloration of nuclei (18) The publications by the University of Pennsylvania workers effectively published the value of oxidant Schiff methods for the histologic diagnosis of fungous infections a principle first recognized by Lillie Their use became virtually routine whenever granulomatous lesions were encountered by the pathologist

But some workers began to prefer the Bauer reaction though inherently less sensitive than the PAS because the weaker or

obtained after such stains as Giemsa's applied to smears or imprints fixed briefly in Zenker formol mercuric precipitates should be removed before staining. When stains for carbohydrates are used fixatives that remove lipids are useful. Brief fixation of the smears in 95% to absolute ethanol methanol or alcohol formalin is preferable to either flaming or air-drying. Flaming of smears is known to be destructive to cytologic details in micro organisms. Air drying also distorts structure and may cause certain lipids to become Schiff positive. Oxidation of unsaturated lipids can produce aldehydes stainable with Schiff's leucofuchsin and conceivably hinder detection of fungi (11)

In smears stained by Wright's or Giemsa's one may suspect histoplasmosis from the finding of macrophages whose cytoplasm is enlarged and stuffed by numerous tiny egg shaped organisms with deeply staining nuclei each surrounded by a halo—the unstained cell wall or capsule (see Fig 1). The organisms vary in size commonly ranging from 1 to 5 microns but most forms are usually 2 3 by 3-4 microns. Exceptionally large forms are seen only occasionally. The greater part of each organism consists of cytoplasm and nonstaining cell wall commonly called a capsule. The chromatin of Hisotplasma seen in smears or imprints is rather complex and variable (6) commonly the chromatin forms an eccentric broken-circle or resembles a pair of parenthesis marks that do not quite touch (26). In paraffin sections the chromatin material of Histoplasma usually appears more simplified and smaller probably as a result of shrinkage during fixation dehydration and paraffin embedding. In smears and imprints protozoa are usually more ovoid or elongate and possess more uniform chromatin than Histoplasma. Recall also the important distinction used by Darling in excluding Leishmania by the failure to find any rod shaped kinetoplasts. In the case of Histoplasma staining by one of the carbohydrate methods to be discussed later will reveal decisive coloration of the cell wall. Such carbohydrate methods would be especially rewarding in the study of smears from older lesions where nuclei of Histoplasma may be scarce or virtually absent. While Histoplasma reproduce in tissues by budding such budding is not usually detectable except when stains coloring the cell wall are used. Features of other yeast like agents are discussed in IV

co workers indicates that birefringence of Histoplasma was especially well seen when stained by methenamine silver after chromic acid They regard birefringence of the organisms as a valuable confirmatory test shown to obtain in most of their cases of histoplasmosis (43 44) Typically such stained Histoplasma showed *Maltese crosses in polarized light Of interest in this connection is* the recent finding by Klatzo and Geisler that the capsules of Cryptococcus neoformans showed much increased birefringence after staining with cresyl violet (16) We have extended these *observations and find that capsules of Cryptococci are brightly* birefringent when stained with any one of numerous metachromatic dyes (35) We also found that Alcian blue staining produces (red *dish) birefringence in the capsules of Cryptococci If of limited* value as a primary means of detecting fungi birefringence may be still useful as a supplementary observation in conjunction with particular staining methods As many artifacts and some elements of pathologic tissues may also show birefringence use of this method requires much care

II THE APPEARANCE OF HISTOPLASMA CAPSULATUM IN SMEARS AND TISSUE SECTIONS

A Smears

Histoplasma can and have been identified in smears made from bone marrow blood sputum or from such accessible lesions as ulcers of the tongue and elsewhere Even in pathologic work more rapid diagnosis of histoplasmosis can be made from the study of smears taken from fresh surgical or autopsy specimens More *prompt recognition of histoplasmosis would allow proof by* cultural studies all too often neglected in human histoplasmosis In the past such smears have usually been examined after such routine stains as Wright s stain the Giemsa or a hematoxylin and eosin But a higher incidence of recognition would be obtained probably by wider use of stains for carbohydrates such as the PAS Gridley stain or chromic acid methenamine silver stain Smears of bone marrow or blood can be prepared in the conventional manner Imprints are made from more solid tissues by touching a freshly cut surface lightly with a clean glass slide Good cytologic detail is

B In Paraffin Sections

In the routine microscopic study of surgical and autopsy specimens Histoplasma are easily overlooked The organisms are not shown adequately by such stains as the hematoxylin and eosin Even in active or early lesions of histoplasmosis organisms are not readily visible except when phagocytosed by macrophages or other cells of the reticulo-endothelial system e g the Kupffer cells of the liver In such cells which may be stuffed literally with Histoplasma the organisms are recognized by their basophilic nuclei and peri nuclear halos representing the unstained cell walls or capsules (see Fig 2) While such forms are highly suggestive better defini tion of Histoplasma results when the cell walls are stained The organisms of older or healed lesions fail to show nuclear staining by hematoxylin as a general rule The shells or cell walls of Histo plasma whose nuclei no longer stain with the H&E can only occasionally be suspected from their faint eosinophilic outlines smaller than those of red blood cells and sometimes showing bire fringence It is now known that Histoplasma are not exclusively intracellular but the extracellular forms when studied in the H&E or other oversight stain are easily confoundable with bacteria nuclear debris and tiny calcific particles Only the nuclei of such forms should be basophilic Staining of cell walls by hematoxylin is exceptional calcified Histoplasma leading to Schaumann like bodies were seen in hamsters by one of us (J K F) but are suspect in other species The reason such organisms are more easily seen in the cytoplasm of phagocytes is that the unstained halo surrounding nuclei of the organisms allows a better suggestion of their cell walls or so called capsules than is likely to occur when organisms are extracellular A bacterial stain such as the Brown Brenn is useful in excluding bacteria because Histoplasma are seldom well stained by such methods We have seen instances of phagocytosed cocci with such thick capsules that they have been mistaken for Histoplasma The recognition of Histoplasma should not depend on the interpre tation of sections stained by oversight methods such as the H&E The various methods for coloring carbohydrates afford incompar ably improved demonstration of Histoplasma as well as the other yeast like pathogens

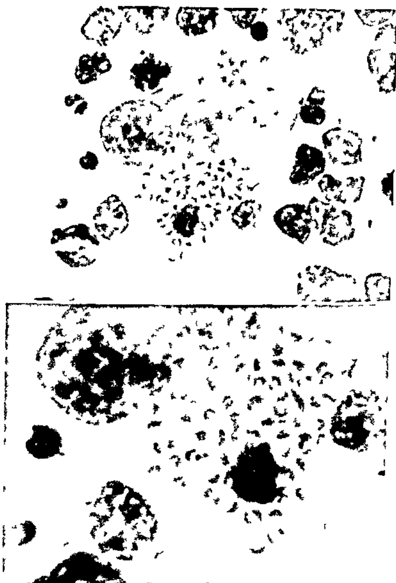


Fig 1 Smear of human bone marrow in disseminated histoplasmosis *Wright's stain* Organisms are seen in the cytoplasm of monocytes as chromatin rich bodies each surrounded by an unstained cell wall (A 1100x B 2100x) In B note that the nuclei of some Histoplasma have the form of bisected spheres

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Fig 2 Section of human (formalin fixed) liver from case of disseminated histoplasmosis *H & E stain* (A 550x B 1050x) Most of the parenchyma in the field is displaced by Kupffer cells whose cytoplasms are gorged with Histoplasma Organisms are seen as rounded dark bodies each surrounded by an unstained cell wall

The presence of high concentrations of carbohydrate in cell walls of Histoplasma was first suggested by Kernohan who mentioned that organisms in one case were colored red by Best's carmine (1943). We have confirmed that the cell walls of Histoplasma are stainable with Best's carmine in nearly all cases (see Fig 3). But the contrast of organisms against tissue obtained with Best's carmine is not as good as that produced by the more modern methods. The use of either Schiff's reagent or Gomori's methenamine silver after oxidation results in stronger coloration of the cell walls and greater contrast between tissue and organisms. When the cell walls are stained, Histoplasma appear larger than in sections stained with just the H & E. The organisms are typically egg shaped and possess distinct walls or shells. There is no capsule external to the cell wall (4) as possessed by Cryptococci. The cytoplasm is usually colored less intensely and sometimes not at all. The nuclear material is often also stained along with the cell walls though seldom so intensely colored. But the coloration of nuclei by the



Fig 3. Section of liver same case as Figure 2. *Best's Carmine stain* (550x). Histoplasma are seen as dense clusters of gray oval to circular bodies inside the cytoplasm of Kupffer cell. The cell wall and some nuclei of Histoplasma are weakly to moderately colored but contrast is poor.

various carbohydrate methods is inconstant. It is often necessary to rely on finding only the typical stained shells. When a basophilic dye is used as a counterstain, nuclei of Histoplasma will be colored if present. In older lesions the organisms possess no definite nuclei. Innumerable Histoplasma are revealed often by the oxidative methods when none are visible in duplicate sections stained with the H & E. Because diverse histological appearances are seen in histoplasmosis (3), such stains should always be used whenever the diagnostic problem of a granulomatous lesion or suspicion of histoplasmosis exists.

The specific advantage of the chromic acid methenamine silver method is that the organisms are intensely stained while the background usually remains colorless. While this may be true, the Gridley method employing Schiff's reagent after chromic acid and certain modifications employing periodic acid (to be presented later) are still considered valuable. The specific recognition of budding is quite helpful but is usually possible only when the cell walls are stained (see Fig. 4).



Fig. 4. Budding forms of Histoplasma revealed by the *chromic acid methenamine silver stain* (1050×). A, B and C are from the same liver shown in previous figures. C shows an exceptionally large budding organism. D, E and F are from an encapsulated caseous adrenal mass in a case showing no definite lesions except in the adrenals; *tissue was fixed in formalin*. Organisms in the latter case almost always lacked nuclei in sections stained by the H & E.

When the cell walls or so-called capsules of Histoplasma are made visible the organisms are more plentiful and no longer appear separated from one another Probably as a result of phagocytosis one often finds multiple individual clusters of organisms whose shells are usually in contact While some individual organisms are seen as isolated forms the tendency to clustering is helpful as such clusters are sometimes visible with low powers of the microscope The most profitable place to look for organisms varies from case to case In the very oldest lesions of the type studied by Puckett and Zimmerman the organisms are seldom seen except in the central part or caseous-calcific core of the lesion (39 16) It is seldom rewarding to look in the fibrous tissue forming the wall of the encapsulated lesion In older descriptions of more active lesions many have said that organisms are found more frequently in the cellular reaction adjacent to necrotic foci This probably means only that macrophages were found at the periphery and allowed intracellular forms to be seen In some cases we have studied the organisms are found largely within areas of caseation In cases of disseminated histoplasmosis organisms may be found throughout both in macrophages and extracellularly or localized in fixed reticulo endothelial cells of such tissues as spleen liver or lymph node Organisms in more active lesions tend to have thinner cell walls and more prominent nuclear material Compared with organisms of more acute or active lesions Histoplasma of older lesions often show cell walls that are unevenly thicker Nuclear material is inconstant and scarce in organisms of very old lesions

As Histoplasma may be very hard to demonstrate in such older lesions it is valuable to study sections cut at various levels Tissue blocks from caseous areas (43 11) and from the central portions of encapsulated lesions are the most apt to reveal Histoplasma While most of the organisms in a given case tend to be uniform some will show considerable variation in size (31 40) Rarely hyphal forms of Histoplasma are seen in tissues (31 47) In one case of histoplasmosis resulting in Addison s disease with severe hypothermia one of us (J K F) saw hyphae in several organs The occurrence of atypical forms of fungi in extensively necrotic lesions is not confined to histoplasmosis and has been noted for example

for Coccidioides immitis (42) Whenever hyphae are seen be careful to exclude the possibility of Candida (Monilia) whose yeast forms are usually associated with septate pseudo hyphae

When the PAS stain is used there is so much background coloration produced that one cannot rely on examining the tissue under low powers of the microscope We have many times found organisms at higher magnifications when such were hardly noticeable at lower magnification It is the fairly *deep* staining of caseous tissue as well as the fibers and cells of chronic granulation tissue that tends to obscure the contrast between organisms and tissue Contrast is better with the Gridley stain since the over all tissue coloration produced by chromic acid Schiff is much less Also the metanil yellow counterstain makes the background yellow while leaving the organisms red As a general rule the various cell granules colored by the oxidative Schiff methods are smaller and more solidly colored while Histoplasma are larger and possess sharply outlined deeply staining cell walls or shells The cytoplasm of Histoplasma is also colored but usually with less intensity While calcific particles are often colored moderately by the oxidative Schiff methods these tend to be non uniform in size and shape and lack the typical shells possessed by Histoplasma Variants of the PAS producing stronger staining of the organisms—to be described —offer less difficulty in the exclusion of other structures and substances While coloration of the nuclei of Histoplasma is helpful when present it is often absent and must not be relied upon

There has been no systematic exploration of the effect of various fixatives upon the stainability of Histoplasma Our own experience indicates that staining is stronger and more consistent in tissues fixed in formalin than by Zenker s or Helly s fixative Based on experience with other polysaccharides alcohol or alcohol formalin fixation should also be most favorable to the preservation of the carbohydrate rich organisms

III TECHNICAL DIRECTIONS FOR THE PERFORMANCE OF METHODS THAT STAIN HISTOPLASMA AND OTHER YEAST LIKE FUNGI IN TISSUES

A General Recommendations

In the staining and study of sections suspected to contain Histoplasma control sections from a case of known histoplasmosis will often prove valuable This allows detection of staining failure should such occur due to deterioration of one or more reagents it provides the less experienced a reference point for the morphology and staining properties of Histoplasma Because Histoplasma are harder to detect than most other fungi it is not extravagant to employ more than one method whenever suspicion of histoplasmosis exists Because Histoplasma are sometimes sparse—especially in older or largely healed lesions—it is helpful to stain sections cut at multiple levels Duplicate or matching H & E s made for some of the additional levels cut allows better correlation in study of the special stains

B The Chromic Acid Methenamine Silver Reaction (Grocott s Application of Gomori s Reaction for Polysaccharides)

In 1946 Gomori introduced a new alkaline silver nitrate reagent that provided selective blackening of some but not all polysaccharides after oxidation with chromic acid (9) He believed that aldehydes generated by chromic acid reduced the silver nitrate to metallic silver Why this did not occur at all tissue sites known to color Schiff s reagent after periodic acid was not clear Despite the general assumption that Gomori s methenamine silver reaction is due to aldehydes the basis of the reaction is not established adequately (23) The same results are not obtained with methenamine silver after periodic acid and some non-carbohydrates may become blackened Despite these problems Grocott in 1955 reported that various fungi were blackened by methenamine silver after chromic acid while the background remained virtually unstained (13) This is a great advantage in photomicrography Grocott s directions differ from Gomori s only in minor details Because alkaline silver reagents are notoriously capricious the worker utilizing them must follow all directions carefully and prepare solutions accurately The

directions that follow adhere closely to those published by Crocott We have found them satisfactory in a number of cases studied by us Specific problems and certain variables of technic are stated at the appropriate steps in the procedure

Reagents for the Chromic Acid Methenamine Silver Reaction

(While these can be re used several times longer limits of safe use have not been determined for each The terms in parentheses are synonyms for the compounds required)

a) *5% chromic acid* (chromic trioxide CrO_3) Dissolve 5 0 gm chromic acid in 100 ml of distilled water

b) *Methenamine Silver Stock Solution* Add 5 ml 5% silver nitrate ($AgNO_3$) to 100 ml 3% methenamine U S P (hexamethylene tetramine $(CH_2)_6N_4$) Shake until the white precipitate initially formed becomes dissolved If stored in the refrigerator (5°C) the stock solution remains clear colorless and effective for months

c) *5% borax* (sodium tetraborate $Na_2B_4O_7 \cdot 10H_2O$) Dissolve 5 0 gm borax in 100 ml distilled water

d) *1% sodium bisulfite* (sodium acid sulfite $NaHSO_3$) This can be made fresh each time by dilution from a more concentrated stock solution e g ten fold dilution of 10% sodium bisulfite made by dissolving 10 0 gm sodium bisulfite in 100 ml distilled water Stopper tightly

e) *0 1% gold chloride* ($AuCl_3 \cdot HCl \cdot 3H_2O$) Prepare from crystal line substance by dissolving 0 1 gm gold chloride in 100 ml dis tilled water or dilute commercial 1% solution of gold chloride ten fold This reagent is expensive but is stable and can be used repeatedly

f) *2% sodium thiosulfate* (hypo $Na_2S_2O_3 \cdot 5H_2O$) Dissolve 2 0 gm of hypo in 100 ml distilled water

Staining Procedure

1a Take sections through xylenes and alcohols to water (Remove mercury precipitates with iodine and hypo when necessary According to Lillie formalin pigment will be bleached during the subsequent exposure to chromic acid (21) We have found that some may persist but can be minimized or removed by pre

liminary treatment in saturated picric acid in 95% alcohol for 15 minutes if alcohol picric is used rinse in 70% alcohol and then in running tap water)

1b Smears should be fixed for 5 30 minutes in 95% alcohol or alcohol formalin rinsed in 70% alcohol and then in water (This also applies to the staining of smears by the other methods to be presented)

2 Rinse in distilled water and oxidize in 5% chromic acid for one hour (Should detachment of sections occur collodionization of duplicate sections prior to chromic acid may prove helpful)

3 Wash in running tap water 10 minutes and then rinse 1 minute in 1% sodium bisulfite Wash in water for 5 minutes Rinse briefly in three changes of distilled water (This thorough washing was intended by Gomori to insure the complete removal of chromic acid)

4 Treat for 60 90 minutes in the *working solution* of methenamine silver at 45 50 C (The working solution is prepared just before use by mixing 25 ml of methenamine silver stock solution with an equal volume of distilled water and 1 ml of 5% borax Grocott said 1 2 ml borax Preheat the working solution to the desired oven or water bath temperature prior to the introduction As the time required for optimal staining varies no fixed duration was prescribed by Gomori He directed that sections be examined at intervals until brown staining by silver seemed adequate Two alternatives seem more expedient Include a *control* section containing Histoplasma after 60 minutes in methenamine silver rinse the *control* in distilled water and examine If Histoplasma are deep brown assume that staining of unknown sections is adequate and proceed to the next step If inadequate in the *control* continue staining for an additional 30 minutes Longer staining has not been necessary But the routine use of methenamine silver for 90 minutes results often in excessive staining The other alternative is to stain two sets of unknowns one for 60 and the other for 90 minutes Discard working solution after each use At the Armed Forces Institute of Pathology (30) the working solution is used at 58°C this produces faster staining but seems less selective and harder to control)

5 Rinse in three two minute changes of distilled water
6 Tone in 0 1% gold chloride for 5 minutes (This tends to bleach the background)
7 After rinsing in distilled water treat in 2% hypo for 2 minutes Then wash thoroughly in tap water
8 Counterstain if desired e g hematoxylin and eosin or 0 1% safranin in 0 1% acetic acid for 5 minutes or 0 05% Light Green S F containing one drop of glacial acetic/100 ml or a brief dip in saturated aqueous picric acid
9 Dehydrate clear and mount in Permount or other synthetic resin (If safranin—a useful counterstain—is extracted excessively by alcohols dehydrate instead in 3 or 4 changes of acetone and go directly to xylenes)

Result Histoplasma are seen as gray to black ovals and spherules with sharp deeply blackened outlines against a background that is colorless except when counterstains are used (see Fig 5)

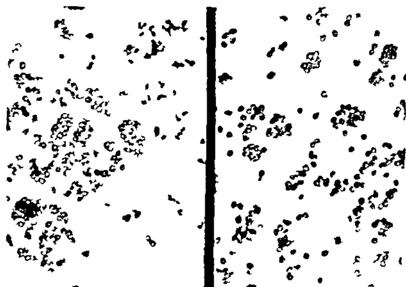


Fig 5 The *chromic acid methenamine silver stain* applied to the liver (A) and to the caseous adrenal mass (B) described in previous figures (550x) The cell walls of Histoplasma are sharply outlined and appear black to gray in the photograph Glycogen granules in liver cells (A) are solidly stained and black As the background is completely unstained contrast of organisms against tissues is excellent

Collagen is usually deep reddish brown and reticulum is often blackened. While certain polysaccharides such as glycogen and epithelial mucins are also blackened, these should cause no difficulties in interpretation. Naturally-occurring pigments, e.g., anthracite and hemosiderin, and particles of dust prove troublesome and must not be mistaken for organisms. For the best definition of Histoplasma avoid overstaining lest the organisms become solidly blackened. Organisms are more distinctive when the shells are blackened but the cytoplasm is less deeply stained. When present, nuclei of fungi are often stained. The duration and temperature of the methenamine silver treatment is the most critical step and determines the depth and extent of tissue blackening. In older lesions counterstains are useful if the organisms are stained deeply enough to provide good contrast. In more active lesions nuclear debris and other basophilic matter can cause confusion when hematoxylin is used, e.g., H & E stain.

Regrettably, erythrocytes are inconstantly colored pink to reddish brown after the chromic acid methenamine silver sequence. Attempts to prevent this by changes in various steps of the method have not succeeded. Compared with Histoplasma, red blood cells when stained appear reddish, less deeply stained and more homogeneous—lacking distinct cell walls.

After chromic acid methenamine silver staining, the organisms show birefringence around the periphery of dense, non-luminous centers giving rise to a "Maltese-cross" appearance (43, 44). Excessive blackening of Histoplasma may obscure such birefringence. The decisiveness and utility of this phenomenon will vary with the equipment and expertness of the microscopist. We observe such birefringence consistently but obtain only faint brightness or contrast with the inexpensive polarizer and analyzer attachments available for conventional microscopes. A polarizing microscope may produce more decisive contrast and increase the utility of this phenomenon.

Comment. This method seems laborious but produces spectacular results in most cases. Additional work is needed to establish the most consistently useful counterstains. At present safranin seems preferable to the H & E stain. It is doubtful that a fixed routine

will prove optimal for all instances Organisms were poorly stained in several cases of Zenker fixed tissues studied by us but results in formalin fixed tissues have been consistently good Specificity of the present method for polysaccharides remains to be proven It is hoped that the method can be simplified and elucidated further

C The Chromic Acid Schiff Reaction (the Bauer Stain and Its Modification by Gridley)

While this reaction is less sensitive than the periodic acid Schiff for polysaccharides in general it is very useful for the detection of Histoplasma and other fungi It is similar to Gomori s reaction except that Schiff s reagent is used instead of methenamine silver Why the silver reagent produces stronger staining of Histoplasma than Schiff s reagent after chromic is not clear Contrast obtained between Histoplasma and the tissue background by Schiff's reagent is reasonably good when organisms are stained deeply enough

This reaction is not specific for fungi but is relatively specific for polysaccharides Chromic acid followed by Schiff s reagent was introduced by Bauer (1933) for the staining of glycogen and later found valuable for epithelial mucins Lillie s discovery (1947) that Bauer s reaction was useful for detecting fungi including Histoplasma (20) was temporarily overshadowed by the general adoption of periodic acid as the oxidant of choice for polysaccharides

Like periodic acid and certain other oxidants chromic acid oxidizes *vicinal hydroxyl groups* i e pairs of OH groups on adjacent carbon atoms to aldehydes Most but not all polysaccharides are stainable with Schiff s after such oxidation Chromic acid produces weaker coloration of most substances in the final result than the PAS This has been attributed to a coarser action of chromic acid viz the degradation of some aldehydes to non stainable derivatives e g carboxyls and carbon dioxide (22) But as fewer tissue structures and substances are stained by the Bauer contrast between tissue background and fungal or other polysaccharides often seems greater than obtained with the conventional PAS

Gridley employed the Bauer stain in combination with Gomori s aldehyde fuchsin found useful for coloring hyphae of

some fungi not well stained by the Bauer alone (12) She also used a metanil yellow counterstain which promotes additional contrast between fungi and tissue This is useful and can be added with profit to the PAS Histoplasma are often more deeply colored in Gridley s variant The reason is not clear as aldehyde fuchsin itself does not color Histoplasma Possibly paraldehyde present in aldehyde fuchsin produces further darkening of bound Schiff s reagent Exposure of sections to formaldehyde after the PAS also deepens the color of some substances (28) The technical procedures are relatively simple and require less time than the laborious chromic acid methenamine silver method Even though fungi are stained less intensely some workers will prefer the convenience of Schiff s over the silver reagent Where resources permit it will prove useful to treat two sets of sections in chromic acid while one set is taken through Schiff s reagent as directed below the other set can be stained with methenamine silver etc with step 3) of the directions given in *B* for the chromic acid methenamine silver method

Reagents for the Chromic Acid Schiff Reaction (and the Gridley Variant)

a) 4% Chromic Acid Solution Dissolve 4 0 gm of chromic acid (CrO_3) in 100 ml distilled water This reagent can be used repeatedly but eventually becomes exhausted Control slides containing glycogen or epithelial mucus should be tested from time to time (For the Bauer stain Lillie (24) uses 5% chromic acid the same strength used for Gomori s methenamine silver reaction This difference in chromic acid concentration probably makes no consistent difference in the final result)

b) Schiff s Reagent Various formulas for Schiff s reagent differ chiefly in the amount of basic fuchsin prescribed and the particular substances generating the sulfurous acid that decolorizes the dye Any Schiff s reagent already giving good results in the laboratory with the PAS can be used probably and might be tried before preparing the particular reagent in use by us (R W M) Combine in order

distilled water	19 ml
conc hydrochloric acid (HCl)	8 ml
sodium sulfite (Na_2SO_3)	0 gm
basic fuchsin (C I 677)	2 0 gm

If not available 3 8 gm sodium metabisulfite ($Na_2S_2O_5$) can be substituted

Insert stopper and shake at 20 30 minute intervals until clear brown to brownish red Add 1 gm active decolorizing carbon e g Norit and shake for 2 minutes Filter through a fine grade of filter paper Filtrate should be clear and colorless While storage in the cold (5°C) is preferable this reagent can be kept at room temperature If large quantities are prepared at one time the reagent keeps better in several smaller well filled bottles instead of a single large container or only partly filled bottles Discard if reagent becomes pink The reagent is fairly stable and can be used many times Exact limits of safe usage must be individually determined by dating the solutions staining of control slides occasionally and some estimation of use frequency

c) *Bisulfite Rinses* A stock solution of molar sodium bisulfite (sodium acid sulfite $NaHSO_3$) is made by dissolving 10 4 gm in 100 ml distilled water This is diluted twentyfold with water just before use Discard the rinses after each use

d) *Gomori s Aldehyde fuchsin Stain (Gridley s Use)* Combine the following

basic fuchsin (C I 677)	1 0 gm
70% ethanol	200 0 ml
conc HCl	2 0 ml
paraldehyde (U.S P)	2 0 ml

Dissolve the dye in alcohol and then add other ingredients Let stand for 2 3 days before use solution turns purple Then store in the refrigerator Filter and warm to room temperature before use This reagent keeps a long while Refilter as necessary

e) *Metanil Yellow Counterstain* Dissolve 0 25 gm metanil yellow (C I 138) in 100 ml distilled water acidified with 0 2 ml of glacial acetic acid (CH COOH) This can be reused a number of times Replace if appreciable black precipitate develops

Staining Procedure

1 Take sections through xylenes and alcohols to water (Remove mercury deposits as necessary or remove formalin pigment if abundant

2 Rinse briefly in distilled water and oxidize in 4% chromic acid for 60 minutes at room temperature

3 Wash in running tap water 10 minutes all color due to chromic acid should be gone

4 Treat in Schiff s reagent for 10 minutes
5 Rinse in three changes of M/20 sodium bisulfite two minutes each Discard rinses after each use
6 Wash 5 10 minutes in running tap water Dip in distilled water
7 Stain in aldehyde fuchsin for 15 30 minutes
8 Rinse off excess stain in 95% alcohol and then wash 3 5 minutes in tap water
9 Counterstain in metanil yellow solution for 60 seconds (While specified by Gridley and found useful by us this can be deleted if wished)
10 Rinse briefly in tap water dehydrate clear and mount

(Note For the original Bauer method omit steps 7 9 and if nuclear staining is desired use Harris hematoxylin counter stain for two minutes followed by the usual rinsing in acid alcohol and water)

Result The cell walls or shells of Histoplasma and other yeasts are colored red by Schiff s reagent (See Fig 6) Nuclear material of fungi unlike those of tissue cells is often Schiff positive when present But nuclear staining of Histoplasma is inconstant Glycogen and epithelial mucins are strongly Schiff positive after chromic but usually easily distinguishable The tissue background is only pale pink unless colored yellow by the recommended metan il yellow counterstain which also colors red blood cells Pseudo hyphae of Candida are well stained by Schiff s reagent after chromic acid but the hyphae of certain other fungi are not

The addition of aldehyde fuchsin i e the Gridley combina tion does not alter the preceding except that Histoplasma are more purplish Also the hyphae of certain other fungi and the acidic polysaccharide capsules of Cryptococci are said to be lavender to deep purple The staining of most epithelial mucins but not gly cogen is deepened to purplish blue possibly owing to additional coloration of acidic groups by aldehyde fuchsin or to action of paraldehyde on bound Schiff s reagent For other specific details consult Gridley s original paper (12) One drawback to the Grid ley combination is the lack of nuclear staining in tissue cells

Comment Although Histoplasma are more deeply colored after the complete Gridley stain than when aldehyde fuchsin is



Fig 6 The *Gridley stain* applied to sections of the liver (A) and the caseous adrenal mass (B) described in previous figures (both 550x) The cell walls of Histoplasma appear as sharply outlined dark gray spherules in the photograph Glycogen granules in (A) are more solidly colored and appear black While the background is colored slightly contrast is reasonably good

omitted the staining of Histoplasma by aldehyde fuchsin is doubted Aldehyde fuchsin used after chromic acid treatment in Schiff s reagent has failed to color Histoplasma Whether aldehyde fuchsin may be used or not the metanil yellow counterstain is advised

D The Periodic Acid Schiff (PAS) Reaction

McManus (1946) (27) and Lillie (1947) (21) independently devised methods similar in essence that utilized periodic acid oxidation followed by Schiff s reagent The PAS proved more sensitive and dependable than older methods for the staining of diverse polysaccharides and other carbohydrates in tissue sections The work of Hotchkiss (14) clarified the chemical basis of the reaction discussed previously The histologic and histochemical value of the PAS was recognized early and its use soon became virtually routine wherever tissues were examined with the microscope Following a series of reports asserting its superiority over the Bauer stain for the detection of fungi the PAS became widely used for this purpose (17 19)

While periodic acid is the oxidant of choice for most naturally occurring carbohydrates of tissues the assumed superiority of the PAS for staining fungi requires qualification As an all purpose method most fungi are colored very well by the PAS reaction But it is usually easier to detect Histoplasma after chromic acid Schiff staining than after the customary PAS technic This is not true of tissue carbohydrates in general Even more important is the difference in contrast between Histoplasma and the tissue background shown by the PAS versus the Bauer or Gridley stains Contrast between Histoplasma and tissue is greater after the chromic acid because the background is usually not colored to any extent by Schiff s reagent Gain in contrast and easier detection of organisms can result either by intensification of fungus staining relative to the tissue background or from the suppression of background staining while organisms remain adequately colored

There are two ways to obtain stronger staining of Histoplasma (and other fungi) by Schiff s reagent following periodic acid oxidation (36) Because these observations are new the interested reader is urged to confirm them by testing sections from one or

more known cases of histoplasmosis before adopting our recommendations That stronger staining could result from more intense oxidation of Histoplasma was observed earlier but evidently overlooked by others (18) In McManus and Lillie s methods sections are treated in aqueous periodic for 5 10 minutes Considerably stronger staining of Histoplasma results if the treatment in aqueous periodic is prolonged to 1 hour Alternatively much enhanced staining of Histoplasma by Schiff s reagent can also be obtained if sections are oxidized for 5 10 minutes in 0 5% periodic acid dissolved *in glacial acetic acid instead of water* Other steps in the PAS sequence require no change The tissue background remains moderately to strongly PAS positive but the use of the metanil yellow step of Gridley s procedure described in *(C)* improves contrast to some extent The proposed changes in periodic acid oxidation apply only to its use in the detection of fungi especially Histoplasma and is not intended for general histological and histochemical usage With few exceptions the numerous other uses of the PAS reaction are served just as well by the traditional brief oxidation in aqueous periodic

Reagents for the Periodic Acid Schiff Reaction (Including Acetic Periodic Schiff Variant)

a) 0 5% Periodic Acid (Aqueous) Dissolve 0 5 gm periodic acid (H_5IO_6) per 100 ml of distilled water Stored solutions keep indefinitely but the working reagent becomes exhausted at a rate that depends on the extent of usage Determine safe limits of usage for the working reagent by occasional checks with control sections that yield good results when all PAS reagents are fresh

b) 0 5% Periodic Acid in Glacial Acetic (Acetic Periodic) Dissolve 0 5 gm periodic acid per 100 ml of glacial acetic acid Periodic acid in acetic is fairly stable and may be used many times The slight amount of white precipitate that forms on standing can be removed by filtering as necessary without evident loss of oxidizing capacity A jar of reagent in use will become exhausted slowly and should be checked at intervals

c) Schiff s Reagent and Bisulfite Rinses See directions already given in the section dealing with the chromic acid Schiff reaction *(C)*

d) Metanil Yellow Counterstain See directions for chromic acid Schiff reaction *(C)*

Staining Procedure

1 Pass sections through xylenes and alcohols to water (Eliminate mercury deposits if Zenker or Helly fixed See directions in *(B)* for removal of formalin pigment when necessary)
2 Select one of the following conditions of oxidation
   a) *traditional* oxidize in aqueous periodic for 10 minutes
   b) *prolonged oxidation* oxidize 60 minutes in aqueous periodic
   c) *acetic periodic* rinse briefly in glacial acetic and oxidize for 10 minutes in periodic acid dissolved in glacial acetic acid (Prior rinsing in acetic prevents watery dilution of acetic periodic)
3 Wash 5 minutes in running tap water rinse briefly in distilled water
4 Treat in Schiff s reagent for 10 minutes
5 Rinse in three changes of M/20 sodium bisulfite 2 minutes each (The special rinses remove excess Schiff s reagent without inducing its spontaneous coloration and should not be omitted) Discard rinses after each use
6 Wash 5 10 minutes in running tap water (Full coloration of stained sites requires adequate washing)
7 *Optional* for the staining of nuclei place in Harris hematoxylin for 30 seconds to 2 minutes (Determine optimal time by experience for your preparation) Dip in water and then briefly in 1% HCl in 70% alcohol until hematoxylin turns reddish Wash 10 minutes in running tap water (Any other contrasting stain that is suitably selective for nuclei can be substituted)
8 *Optional (but recommended) metanil yellow counterstain* dip in metanil yellow for 60 seconds Wash briefly in tap water
9 Dehydrate clear and mount

Result Tissue structures and substances that react with the PAS are colored magenta The strongest reactions or depth of color occur in the cell walls of fungi glycogens epithelial mucins renal basement membranes and brush borders and other sites believed to contain carbohydrate or glycoprotein (As lipids may interfere in frozen sections of fresh tissue or in smears that have not been defatted specificity of the PAS for carbohydrates applies more strict



Fig 7 *Periodic acid Schiff reaction* applied to sections of liver (A 550x) and the caseous adrenal mass (B 1050x) described in previous figures In A oxidized for ten minutes in 0.5% periodic acid organisms are stained fairly strongly and glycogen granules appear black but contrast of organisms against tissues is poor owing to background staining In B oxidized in periodic acid for five minutes organisms are barely detectable even at high magnification

ly to its use on paraffin sections) Collagen and reticulum are moderately PAS positive and occasionally strongly colored Nuclei of tissue cells and their cytoplasm when devoid of PAS positive granules should be uncolored except by counterstains when used Red blood cells are colorless unless stained by metanil yellow Some bacteria e g pneumococci are PAS positive and must not be mistaken for the Histoplasma which are larger

The cell walls or shells of Histoplasma will be colored after any one of the oxidation schedules given but their intensity or depth of color is only slight to moderate after the *traditional (brief) oxidation in aqueous periodic* (See Fig 7) Granules of glycogen other PAS positive cell granules and some pigments tend to be finer in size and colored solidly contrasted to Histoplasma which show strongly colored shells with paler cytoplasm Russell bodies are solidly colored and associated with plasma cells The staining of Histoplasma nuclei is fairly constant in active lesions believed to contain live organisms Nuclear staining of Histoplasma in old lesions is inconstant and often lacking The *prolonged oxidation (aqueous) or oxidation in acetic periodic produces* decisively stronger staining of cell walls of Histoplasma but fails to cause any stronger staining of their nuclei (36) (See Fig 8) Usually pathogenic yeasts other than Histoplasma are colored adequately after the traditional oxidation but contrast of organisms against tissue is more pronounced after the acetic PAS As the caseous necrotic background of old or fresh lesions is usually moderately to strongly PAS positive organisms may not be readily visible except at higher magnifications (high-dry) of the microscope The metanil yellow counterstain helps to alter the background color to orange red and improves contrast to some extent Sections should not be thicker than 6 8 microns or dense staining may obscure organisms

A nuclear stain e g hematoxylin is generally useful especially in the study of older lesions But in some active lesions the presence of abundant deeply staining nuclear debris may hinder the detection of Histoplasma unless the latter are also abundant and deeply stained Do not persist in the use of a hematoxylin routine that causes cytoplasmic basophilia after the PAS Excessive or poorly selective hematoxylin can spoil an otherwise satisfactory PAS stain

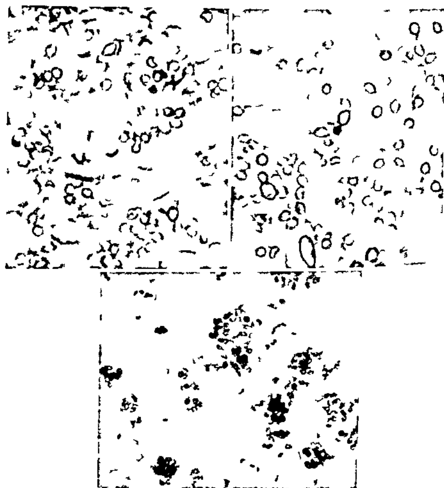


Fig 8 *Effects of changes in the use of periodic acid on the staining of Histoplasma by Schiff s reagent* A and B (each 1050x) are duplicate sections of the caseous adrenal mass shown previously The section shown in A was oxidized for sixty minutes in 0 5% periodic acid while that in B was treated in 0 5% periodic in glacial acetic acid for just five minutes Section C (550x) of liver shown previously was oxidized for ten minutes in 0 5% periodic acid in glacial acetic acid Organisms are more deeply stained by Schiff s reagent than after oxidation in aqueous periodic acid for five to ten minutes Compare with Figure 7 and other figures

Comment The chief obstacle to the detection of Histoplasma by the PAS reaction is the fairly strong coloration produced by other tissue components especially in the caseous necrotic material or in the collagenous partly calcific content of older lesions Background staining by Schiff s reagent is much reduced without impairment of Histoplasma coloration if sections are placed in 5% chromic acid for thirty minutes (and washed) following initial oxidation by prolonged periodic or by acetic periodic acid (35) Compared with Histoplasma calcific particles tend to be less uniform in size and moderately stained in toto without sharply defined shells Stained objects other than fungi cause less trouble in the study of more active lesions or viscera involved during disseminated histoplasmosis

IV CYTOLOGIC AND CYTOCHEMICAL FEATURES OF OTHER YEAST LIKE MICROBIAL AGENTS

Because Histoplasma are small and rather non-descript even when nuclei are present presumptive histologic diagnosis requires the exclusion of other yeast like agents in the differential diagnosis Many of these agents can be identified more reliably than Histoplasma The fungi of the group are usually well stained by oxidative reactions for polysaccharides Criteria found useful for recognition of these agents will be reviewed briefly Of course confirmation by micro biological methods should be attempted whenever possible

A Blastomyces dermatitidis

This agent provokes a partly suppurative and partly granulomatous inflammatory reaction (1) The organisms occur in tissues as thick walled budding yeasts that normally range from 5 30 microns in size but most forms are 7 20 microns The organisms possess relatively large round nuclei stainable with either basic dyes or by oxidative reactions The cell wall is described as doubly contoured after routine stains but is usually solidly colored by oxidative reactions Buds are usually single and have a wide stalk While a thin rim of acidic material (37) usually less well colored by oxidative reactions is occasionally seen external to the cell wall

Blastomycetes possess no mucinous capsules like those of Crypto cocci There is usually a space often lunar shaped between the cell wall and the nuclear material (1) Some microforms (2-4 mi crons) are not uncommon and may predominate in exceptional cases (31) These will be difficult to distinguish from Histoplasma except that pathologic features of the two diseases differ as a rule Compared with Histoplasma cell walls of such small Blastomy cetes are usually thinner and nuclei larger and more constant Both may show similar birefringence after methenamine silver (35)

B Blastomyces brasiliensis

The general features are similar to B dermatitidis except that multiple buds arising from filamentous stalks occur Microforms also occur (31)

C Cryptococcus neoformans

There may be virtually no inflammatory reaction ranging to chronic inflammation that is granulomatous to varying degrees (25) The organisms occur as thin walled budding yeasts possess ing as a rule mucinous capsules rich in acidic polysaccharide (37) They range in size from 2 20 microns but most will be 5 15 microns The capsules are usually thick especially when mounted in glycerin jelly (33) after the Alcian blue (32) or our colloidal iron stain (34) Mucicarmine is much used for capsule staining (1 25 41) but is less sensitive than the newer methods mentioned But some strains have less thick capsules and the smallest forms commonly lack them While small inclusions are sometimes present the organisms lack the large nuclei regularly seen in Blastomycetes The intensity of staining by oxidative methods of polysaccharides depends on the extent to which the capsules are present and stained Buds usually have slender stalks Germinal tubes consisting of 3-4 consecutive and undetached buds are sometimes mistaken for hyphae Con fusion with Histoplasma should occur but rarely if care is taken to reveal capsules in Cryptococci

D Coccidioides immitis

The usual tissue response varies In active lesions it is partly suppurative with a tendency to granulomatous reaction that be comes dominant in older lesions often with caseation and tubercles

(141) Depending on the site and duration of the disease various forms of the organism may occur in tissues The key diagnostic feature is the presence of the reproductive form viz the endosporulating spherule which may range from 15-75 microns in size and larger These contain a varying number of young forms called endospores (2.5 microns in diameter) Rupture of spherules releases free spores that gradually enlarge and eventually form reproductive spherules Free spores vary in size up to 50 microns or so and closely resemble Blastomycetes except that budding is lacking with rare exceptions (5) Also the internal material of spores tends to fill the cells or coat the inner surface of the cell wall thereby differing from Blastomycetes (1) the internal material of such spores is basophilic stainable by oxidative reactions and partly removable by diastase The cell walls are thick and deeply stained by such oxidative reactions In cavitary lesions of the lung hyphae with arthrospores are frequent often with scarcity of spherules (142) Hyphae have not been seen elsewhere Only the endospores and young free spores are likely to cause confusion with Histoplasma Presence of endosporulating spherules resolves this problem

E Candida (Monilia various species)

Detection of these organisms is very frequent While saprophytic growth is the usual explanation primary infection with chronic or even granulomatous inflammation may occur Organisms occur in tissues as oval thick walled budding yeasts ranging from 1-6 microns in size but usually 2-4 microns These are usually associated with a variable number of pseudohyphae that are septate and sometimes branching Rounded spores similar to the free yeasts may occur at points of constriction in the pseudohyphae The yeasts and pseudohyphae are dependably Gram positive and stained more deeply than Histoplasma by oxidative reactions The yeasts seem to be stained solidly their thick cell walls obscuring nuclear detail Distinction from Histoplasma also depends on histological assessment of the lesion and on the presence of pseudohyphae hyphae are rare in histoplasmosis

F Sporotrichum schenckii

As histological detection of this organism is still regarded as a rarity confusion with Histoplasma seems unlikely The histologic

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D Coccidioides immitis

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REFERENCES

1 Baker R D D agnos s of fungus diseases b, biops, *J Chron Dis* 5 5? 0 13 ,

2 Bauer H Mikroskopisch-chemischer Nachweis v n Glykogen und einigen anderen Polysacchariden *Ztschr M kr an t Fors I* 33 143 160 1933

3 Binford Chapman H H toplasm st ti e reactions and morph logic varia ti n of the fungus *Am J Clin Path* 5 2, 3(19 5

4 Conant N F Sm th D T Baker R D Callaway J L and Mart n D S *M nual of Clin al Myc l gy* ? , 1 F 1 Philadelphia Saunders 19 1

5 Creitz J R Atypical tis ue f rm of *Coc i l ides immitis* resembling blasto myces *An J Cl n Path* 6 l,,1 1950 19,6

6 Darling S T Hi toplasmosi a fatal infect s d sease resembl ng kala azar found among natives of tr p cal America *J l Int Me l* 10 1 5 1909

7 Frenkel J K Path genesi of toxopla m s *An N w York Acad Sc* 64 ?1 2,1 19,6

8 Frenkel J K and Friedlander S *Toxopla mo s Pathol gy of Neonatal D s ea es Pathog n s s D agnos s a d T eatment* Pub No 141 U S Pub Health Service 1) 1 pp 10

9 Com ri G New h tochemical test for glyc gen an l mucin *Am J Clin Path Techn al S ct on 10* 1 1 9 1,46 (Acc mpanying Vol *16* of *Am J Cl n Path* 1946)

10 Gom ri C Aldehy le fu h n a new sta n for ela tic t sue *A n J Cl n Path* 0 6(,666 19 0

11 Comori G *Microscop c H stochem try* Chicago Uni Ch cago Press 19 ? ? 3 pp

12. Gr dley M F A tain for fungi in tis ue sect ons *A J Cl P H* 3 303 307 19 3

13 C roc tt R C Sta n for f ngi in ti u sect n and smears u ng Gomor s methenam ne s l er nit ate techni *An J Cli P th* 3 9, 9 9 1)

14 Hot hki s R D Mi rochemi al react on resulting in the staining of polysac chari le struct res in f xed t ue prep rations *A ch B och m* 16 131 141 1948

15 Kernohan J W A new stain ing meth od to dem nstrate pathogenic ea t and fun i *J Neu opath & F per Neur l* 9 93 1933

16 Klatzo I an l Ceiler I H Demonstration of *Crypt coccus n f rm ns* in polariz ed l ght *Sta n Techn l* 33 5 5(19 8

17 Kl gman A M The d agnosis of fungus infecti ns w th part cular referen e to sta ing methods In *Pr g ess in Fun lam nt l Med ci e* McMa t J F A ed Philadelphia Lea 19 ? Chap 4 pp f9 90

18 Kl gman A M and Bal lr dge G D Morph logy f *Spo t l l k* and *Histoplas na caps latum* in tissue *A M A A ch P th* 51 ,6 4 13 1

19 Kligman A M and Mescon H The pe od a l Schiff ta n f th demon strat on of fungi n a mal tissues *J B t 60* 41 4 1 19 0

20 Lillie R D Reaction of ar ous p ra t organi ms n ti ues to the Bauer Feulgen Gr m and Gram Weigert methods *J Lab & Cl n Med* 3 6 88 191

1 Lillie R D Reti ulum stain ng w th Sch ff reagent after ox lat on by aci lified sodium pe odate *J Lab & Cl n Med* 3 910 91, 1947

reaction is usually pyogenic and necrotizing with a variable granulomatous component (4) The typical clinical infection usually leads to suspicion and isolation of the agent by other means But use of the more sensitive oxidative reactions now favored for Histoplasma should reveal Sporotrichum more frequently Also the moderate coloration seen in the walls of Sporotrichum in mice after stains for acidic polysaccharides (25 37) can be combined with oxidative Schiff s staining with intensification of their net coloration (35) In mice the fungi are 3 5 microns and cigar shaped

G Other Microbial Agents

Phagocytosed cocci with thick capsules may sometimes be mistaken in H & E stained sections for Histoplasma (35 37) Gram type stains the nature of the lesion and the smaller size of cocci should prevent this

Toxoplasma occur in tissues in two forms viz the *proliferative* organisms typically plentiful in active cases seen in newborn infants and in *pseudocysts* that are larger and typical of more chronic infections (7 8) The former are crescentic usually with one rounded end and measure about 3 x 5 microns in smears but smaller in sections The proliferative organisms possess basophilic nuclei and a few glycogen granules Diagnosis from these forms alone is difficult except by assessment of the clinicopathologic features present Pseudocysts are more distinctive and allow safer diagnosis These range from 30 100 microns in diameter and usually contain young forms each with a prominent glycogen granule about the size of the nucleus The cyst wall is stained by oxidative reactions and is argyrophilic Tissue cells filled with proliferative organisms may be confoundable with pseudocysts but lack the distinct walls of the latter

Pneumocystis carinii is regarded as the cause of a distinctive plasma-cell pneumonia seen largely in infants These are yeast like organisms (2-4 microns) seen better in smears than in tissue sections The bodies are not revealed decisively except by the Gridley stain or by methenamine silver after chromic acid Whether confoundable with Histoplasma or not remains to be seen

44 Sweany H C. Gorelick D Collier F C., and Jones J I Pathologic findings in benign pulmonary histoplasmosis Pt II *Dis Chest 34* 257 73 1958

45 Weed L A North American blastomycosis *Am J Clin Path* 5 3 1 19

46 Zimmerman L. E Demonstration of Histoplasma and Coccidioides in so called tuberculomas of lung *A M A Arch Int Med 94* 690-699 19 4

4 Zimmerman L. E Some contributions of the histopathological method to the study of fungus diseases *Tr New York Acad Sc Ser II 19* 358 371 19

22 Lillie R D Histochemical comparison of the Casella Bauer and periodic acid oxidation Schiff leucofuchsin technics *Stain Technol* 26 123 136 1951

23 Lillie R D Argentaffin and Schiff reactions after periodic acid oxidation and aldehyde blocking reactions *J Histochem* 2 127 136 1951

24 Lillie R D *Histopathologic Technic and Practical Histochemistry* New York Blakiston 1951 501 pp

25 Littman M L and Zimmerman L E *Cryptococcosis* New York Grune 1956 205 pp

26 McLeod J H Emmons C W Ross S and Burke F G Histoplasmosis *J Pediat* 28 2[illegible]5 295 1946

27 McManus J F A Histological demonstration of mucin after periodic acid *Nature* 158 202 1946

28 McManus J F A Personal communication to the authors

29 McManus J F A and Mowry R W Effects of fixation on carbohydrate histochemistry *J Histochem* 6 309 316 1958

30 *Manual of Histologic and Special Staining Technics* Armed Forces Inst Path Washington D C 1957 206 pp

31 Moore M *Morphologic variation in tissue of the organisms of the blastomycoses* and of histoplasmosis *Am J Path* 31 1049 1063 1955

32 Mowry R W Alcian blue technics for the histochemical study of acidic carbohydrates *J Histochem* 4 407 1956

33 Mowry R W Value of mucopolysaccharide hydration in the demonstration of microbial capsules in paraffin sections with special reference to *Cryptococcus neoformans* and pneumococci *Am J Path* 34 595 596 19 8

34 Mowry R W Improved procedure for the staining of acidic polysaccharides by Muller s colloidal (hydrous) ferric oxide and its combination with the Feulgen and periodic acid Schiff reactions *Lab Invest* 7 566 576 1958

35 Mowry R W Unpublished work

36 Mowry R W and Hooper C S Unpublished work

37 Mowry R W and Winkler C H Coloration of acidic carbohydrates of bacteria and fungi in tissue sections with special reference to capsules of *Cryptococcus neoformans* pneumococci and staphylococci *Am J Path* 3 6 8 1956

38 Potenza I and Feo M Use of polarized light in diagnosis of mycotic infections *Am J Clin Path* 6 545 5 1 1956

40 Schwarz J Pathogenesis of histoplasmosis *Tr New York Acad Sc* Ser II 0 541 548 19 8

41 Schwarz J and Baum G L Surgical pathology of fungus diseases *A M A Arch Surg* 75 41 56 1957

42 Puckett T F Hyphae of *Coccidioides immitis* in tissues of the human host *Am Rev Tuberc* 70 320 327 1951

43 Sweany H C Gorelick D Cooler F C and Jones J L Pathologic findings in benign pulmonary histoplasmosis Pt I *Dis Chest* 34 119 137 19 8

[illegible] numbers in the text

pearance of exudate When the inflammation remained acute without a quiescent period a severe nodular iritis occurred in one to two weeks Within two to four weeks more the severe inflammation had begun to disappear and became quiescent in two to three months Nodular scars remained after the inflammation had subsided Heat killed spores would not produce an iritis

Microscopic sections of the eyes during the first week after infection showed a few intact spores and mycelial elements The amount of exudate varied with the size of the infecting dose The exudate was comprised of many polymorphonuclear and mononuclear leukocytes and few macrophages containing yeast organisms Rare giant cells and occasional epithelioid cells were also present As the nodules appeared the microscopic lesions became granulomatous characterized primarily by round cell infiltrates and epithelioid cells Yeast cells were prominent within macrophages and some of the many giant cells A few intact spores could even be seen as long as 2½ weeks after infection Abscesses with tissue necrosis were present four to six weeks after infection By two to three months there was gradual subsidence of the inflammatory process Yeast cells could then rarely be seen in phagocytes although the organism was culturally proved to be present in the eye of one rabbit six months after infection Giant cells were absent and only a few epithelioid cells persisted The infiltrates were composed extensively of the round cell type The course of the uveitis was apparently self limiting

The early tissue reaction and healing of lesions in guinea pigs and gerbils produced by the localized subcutaneous inoculation of pure yeast forms pure mycelial elements spores and mixed cultures of Histoplasma capsulatum were even better defined microscopically by Brandt (2) During the first 24 hours following the subcutaneous injection of pure yeast forms into the abdominal and vaginal wall of guinea pigs yeast organisms remained extracellular A large number of leukocytes but very few histiocytes made their appearance By 48 hours the leukocytes were fewer and macrophages which had increased in number had engulfed many of the histoplasma yeast cells By the sixth to tenth day a central area of acute inflammation had developed Many pus

THE PATHOGENESIS OF HISTOPLASMOSIS IN ANIMALS

JOHN J PROCKNOW

The natural route of infection with the fungal organism Histoplasma capsulatum is quite generally considered to be the respiratory tract Animals like man are assumed to acquire a primary pulmonary infection by inhalation of air borne macroconidia produced by the fungal saprophyte growing freely in the soil After lodging in the lung parenchyma the inhaled histoplasma spore would be expected to convert to the parasitic yeast like form Dissemination of the histoplasma yeast via the bloodstream and subsequent parasitization of organs and tissues rich in reticulo endothelial cells such as the liver spleen lymph nodes bone marrow and adrenals would be expected to follow The many studies of naturally occurring and laboratory induced infections in different species of animals must be integrated to satisfactorily postulate the pathogenicity of histoplasmosis

The immediate response of the host to the presence of the infecting fungal agent in either the mycelial or yeast form has been demonstrated locally in various extrapulmonary tissues of animals Although these tissues are unnatural sites of entry for Histoplasma capsulatum the response to the infecting organism parallels and substantiates studies of pathogenesis in the lungs of experimental animals

Day (1) induced a localized infection in the anterior chamber of the eye of the rabbit and was first to show the early development and healing of the granuloma of histoplasmosis A destructive nodular anterior uveitis resulted from the injection of a saline suspension of viable histoplasma spores into the anterior chamber of normal rabbits Grossly dilatation of vessels and exudation appeared in the iris within 24 hours The immediate inflammation subsided in some rabbits within a week Nodules appeared in two to four weeks and subsequently became larger with the reap

macrophages containing yeast forms located close to the area of necrosis

The pathogenesis of histoplasmosis has been best documented experimentally in the white mouse although the sequential pattern of the disease must be gleaned from many studies De Monbreun (4) and other workers (5 8) demonstrated the susceptibility of the laboratory mouse to infection with the mycelial and yeast forms of Hystoplasma capsulatum The reliability of the mouse as a tool for the isolation of the fungus from tissues (9 11) or from soil (12 14) substantiated its predictable infectivity Susceptibility to infection with variable strains and infective doses of yeast phase Histoplasma capsulatum by the intracerebral (15 19) intravenous (6 18 20) intraperitoneal (18 19 21 24) and intranasal (19) routes has been demonstrated in the white mouse The mycelial phase as an inoculum has also been highly successful intraperitoneally (1 7 22 25 28) intravenously (6 8 29) and intranasally (27 28 30) in producing a recognizable infection in mice proved culturally and pathologically Following infection a generalized histoplasmosis quite uniformly develops frequently resulting in death but also tolerated as a chronic infection The disseminated histoplasmosis produced in mice directly exposed to naturally contaminated soil by inhalation of the dust aerosol shown by Hinton *et al* (31) strongly emphasizes that the animal becomes infected in nature primarily via the respiratory route

In our studies at the University of Chicago (30) the very early pathogenesis of experimental histoplasma infection of pulmonary tissue has been well delineated in the white mouse The progressive pathologic cellular reaction to the histoplasma spore within the mouse lung has been observed from early implantation through its subsequent rupture and conversion to the yeast phase The natural mode of infection was simulated by the intranasal inoculation of a saline suspension of viable mature tuberculate chlamydospores It was culturally demonstrated that dissemination of the yeast organism from the lung to the spleen and liver had occurred within four days By the end of two weeks heavy and widespread reticulo endothelial involvement had developed Microscopic examination of the lungs of the spore infected mice were

cells occasional histiocytes and lymphocytes comprised this area Surrounding it were reticulo endothelial elements interspersed with a few polymorphonuclear leukocytes lymphocytes plasma cells and Langhans type giant cells Fibrous tissue in turn surrounded this cellular accumulation Yeast forms remained free within the acutely inflamed area while many others were contained within the reticulo endothelial and giant cells Healing had begun by the twelfth day characterized by an increase in plasma cells *the appearance of fibrocytes and decrease in reticulo-endothelial cells* Healing continued through the 31st day although the yeast cells were few to absent by the twenty first day

When pure spore free mycelial elements were inoculated subcutaneously there was conversion to the yeast phase within one week The cellular response was similar to that induced by pure yeast organisms Fragmentation of the filaments and ingestion by reticulo-endothelial cells occurred before the typical phagocytized yeast forms were present at the end of a week

Following the subcutaneous inoculation of a suspension of pure chlamydospores many spores were phagocytosed intact by the large abundant multinucleate giant cells during the first few days Reticulo endothelial hyperplasia was evident Other free spores became completely surrounded by a ring of polymorphonuclear leukocytes After the first week most of the spores had begun to show loss of chromatin network as they underwent degeneration although the tubercles and spore wall of some remained intact for a longer time In other spores there was an internal rearrangement clumping and division of nuclear material to form yeast cells With the ultimate rupture of the thick spore wall the yeast organisms were released into the tissue where they were engulfed by macrophages There was no systemic spread of the infection into other organs

A more marked local subcutaneous reaction to mycelial organisms was shown in rabbits by Scheff (3) An inflammatory focus with central necrosis developed within 48 hours after inoculation Microscopic examination of the excised lesion on the fifth day showed marked leukocytic and monocytic cellular infiltration Tissue destruction was present in the necrotic area with a few

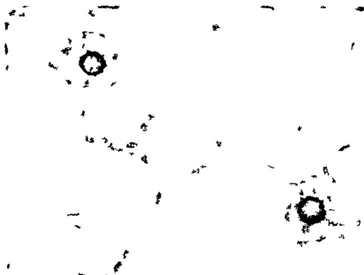


Fig 2 Increased localized inflammatory reaction to spores located within alveoli in lung of mouse 15 hours after infection (x700—Hotchkiss McManus)

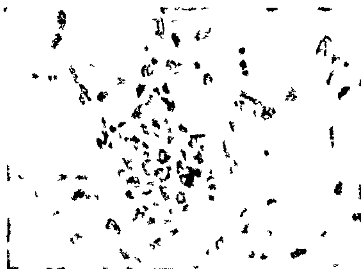


Fig 3 Yeast containing macrophages within and surrounding focal exudate in lung of mouse 36 hours after infection (x1000—HE&A)



Fig 1 Tuberculate chlamydospore with slight cellular response in lung of mouse three hours after infection (x700—Hotchkiss McManus)

made as often as three hour intervals following inoculation The spores were usually small enough to pass deeply into the bronchioles or directly into many alveoli

From three to six hours after inoculation spores were seen in the alveoli and bronchioles without any or only slight cellular reaction to the individual spores (Fig 1) At most only a few polymorphonuclear leukocytes ringed the spores in this early phase The cellular infiltrates about the spores progressively increased in size thereafter Polymorphonuclear leukocytes remained the prominent cell type By 15 to 24 hours after inoculation the inflammatory reaction had become much more extensive though still highly localized The spores remained remarkably preserved and characteristically distributed in alveoli or in terminal bronchioles (Fig 2)

By 36 hours after infection many spores still maintained a remarkable state of preservation The polymorphonuclear leukocytes had begun to degenerate and to be replaced by epithelioid cells The exudate had increased to fill neighboring alveoli and

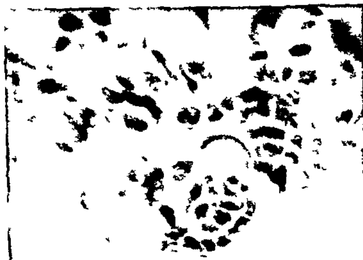


Fig 5 Spore wall fragments being phagocytosed but still present in lung of mouse 7 days after infection (x1400—Hotchkiss-McManus.)

capsules were surrounded by polymorphonuclear leukocytes and engulfed by macrophages Disintegration of the intact spores and release of the parasitic yeast continued until the seventh day by which time all spores had disappeared Only minimal amounts of spore wall fragments remained to be phagocytosed (Fig 5)

By the end of one week focal granulomatous infiltrates had clogged many alveoli adjacent to the bronchioles Macrophages completely engorged with encapsulated histoplasma yeast cells had become numerous and were scattered throughout the exudate However in other densely cellular areas round cells and epithelioid cells were much in evidence while yeast filled macrophages were extremely few By ten days following inoculation widespread pulmonary infection with Histoplasma capsulatum was present characterized by diffuse foci of dense exudative response throughout the lung fields (Fig 6) Many mice had begun to expire of disseminated histoplasmosis at this stage of the infection and lesions were present in other organs of the reticulo endothelial system

Grayston *et al* (27 28) also showed that discrete lung lesions

macrophages were present within the exudate A few were parasitized by encapsulated histoplasma yeast cells Particulate matter probably composed of hypal or spore wall elements was found interspersed through some areas of exudate (Fig 3) No spores were found to be phagocytosed intact by macrophages

At 48 hours an internal trabeculation of the histoplasma spore now almost entirely surrounded by epithelioid cells appeared This internal segmentation was more clearly seen during the next 12 to 24 hours The chitinous capsule of the spore remained intact and the tubercles were prominent about the periphery

By the third to the fifth day following infection the walls of the spores were generally smooth and defects became apparent With rupture the yeast like contents bulged through these defects and were exuded into the parenchyma to be ultimately phagocytosed by macrophages (Fig 4) Occasionally discrete globular masses of well encapsulated yeast cells surrounded by epithelioid cells could be seen in identical position in the exudate in which identifiable spores were observed earlier Remnants of the spore

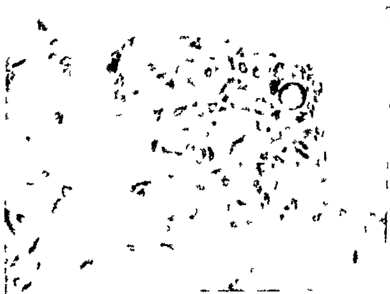


Fig 4 Histoplasma spore rupturing and protoplasmic content bulging through defect in lung of mouse 78 hours after infection (x700—Hotchkiss McManus)

present The initial presence of the yeast or spore and its fragments has attracted the larger number of polymorphonuclear cells The macrophages appeared particularly when the yeast organisms were released into tissue By comparison the typical progressive reactivity of the mouse lung to the presence of the yeast phase of Histoplasma capsulatum inoculated intranasally was demonstrated by Grayston and Salvin (19) Small infiltrates of lymphocytes and macrophages involving only a few alveoli appeared two days after infection The infiltrates were again peribronchial in location but contained only a very few polymorphonuclear leukocytes The lesions increased in size during the first week and remained composed of round cells with some macrophages containing the histoplasma organism By fourteen days the lesions were quite localized Subsequently there was healing by resolution Epithelioid granulomas surprisingly did not develop in these experiments By two to four months after infection infrequent resolving lesions persisted but the lungs continued to show increased lymphatic tissue particularly in the area of blood vessels Round cell infiltrates appeared in the livers by the fourth day after infection There was progressive maturation of the typical hepatic granulomas by fourteen days with subsequent healing and resolution No other pathologic changes were found in other organs

With the conversion of the spore to yeast organisms and phagocytosis already apparent in our study by the second day following infection the bloodstream should obviously next serve as the medium for the systemic spread of the parasite Cultures of the spleen and liver showed increasing positivity and more heavily infected tissue by four days after intranasal infection attesting to an early secondary hematogenous spread At the end of two weeks the blood was frequently positive for Histoplasma capsulatum by culture The numbers of organisms *in the lung tissue had steadily* increased to a maximum by ten days following infection Optimum seeding of organisms into the blood probably took place during this period of propagation The exponential growth of the organism in tissues during the first and second weeks following infection with sublethal doses of yeast organisms intravenously has been further explored (20 25) After one week the number of organisms



Fig 6 Diffuse focal granulomas throughout the mouse lung 10 days after infection (Low power—HE)

of considerable size were definitely present by one week following intranasal inoculation of spores These lesions were discrete and peribronchial in location and tended to coalesce in the hilar region Progression of the lesion after the first week was slight The lesions were composed of mononuclear infiltrates rare polymorphonuclear elements and no edema Yeast like organisms were never abundant within these lesions and they disappeared rather rapidly Atelectasis was noted about the larger confluent hilar infiltrates By one month epithelioid cells had replaced the acute reaction Some lymphocytes and macrophages remained There was neither necrosis nor fibrosis There was a tendency for the lesions to disappear eventually Frequently infiltrates of round cells were present about the pulmonary vessels Pulmonary lymphatic tissue hyperplasia was marked Granulomatous organization resolution and even disappearance of lesions occurred within two to four months

The very early tissue response to the spore or yeast has varied in regard to the numbers of the polymorphonuclear leukocytes

introduction of yeast like organisms into the experimental animal by any route there is usually a prompt local response to the inoculum a generalized proliferation of the cells of the reticulo-endothelial system and engorgement of these cells with the yeast organism of Histoplasma capsulatum This hyperplasia of the reticulo-endothelial system and infiltration with cells of the monocytic and macrophagic type both containing large numbers of the parasites have been noted in the lungs spleen liver lymph nodes bone marrow adrenals heart kidney brain pancreas testes gastrointestinal tract and subcutaneous and connective tissue of infected mice When intravenous injection of the yeast or mycelial form was performed disseminated histoplasmosis resulted regularly Animals autopsied when death occurred spontaneously or late in the course of the disease have provided the pathologic sections used in describing the typical disseminated granulomas (6 8 29 32)

Microscopic sections of the lungs showed hyperplastic and heavily parasitized cells filling the interalveolar septa and collapsing some of the alveolar spaces Occasional parasitized cells were found free in alveoli It is difficult to understand why Baum *et al* (29) found the lungs uninfected in their study

Within the liver and spleen small infiltrates of round cells increased in size during the first two weeks The proliferated Kupffer cells of the grossly enlarged liver were found heavily parasitized distending the sinusoids and compressing the cells of the liver cords to the point of necrosis in some areas Generally the liver cords were preserved however Some polymorphonuclear leukocytes surrounded areas of necrosis Infiltrates were frequently noted in the vicinity of central veins Multinucleated giant cells appeared by the end of one month Phagocytes lymphocytes and a few plasma cells were interspersed in the connective tissue of the portal canals Yeast cells were occasionally seen lying free within the tissue By four to eight months liver parenchyma was usually replaced by coalescent granulomas and macrophages and giant cells containing an abundance of yeast cells In the enlarged spleen the structure was distorted by marked hyperplasia of the phagocytic reticulo endothelial cells lining the sinusoids and containing many yeast organisms Normal lymphoid follicles were often maintained Caseation necrosis was present in severely invaded areas Macro-

recoverable by culture from the tissues became stationary or decreased After three to four months tissue counts were 100 to 1 000 fold less than during the first and second weeks following infection The growth pattern of the histoplasma organism was similar in the spleen and liver during the course of infection There was some delay in the presence of organisms in lung tissue indicating secondary infection The presence of the organism in the blood was not proved until the second week after infection In another study (24) following intraperitoneal infection with yeast phase organisms cultures were often positive from the reticulo-endothelial tissues as late as 45 weeks after infection although the lungs and heart s blood were usually negative by culture within 16 weeks after infection Fungus has been found persistently following infection for an eight month period of observation (27)

By using the intravenous route for experimental inoculation of the yeast form of Histoplasma capsulatum the pathogenesis of disseminated histoplasmosis should be simulated Very few organisms are necessary to establish such an infection Rowley and Huber (33 34) have shown that a single viable yeast like aggregate was sufficient to infect a mouse intravenously Histoplasma infections could be regularly and uniformly induced by the intravenous injection of approximately 100 aggregates per mouse regardless of the strain of the organism Differences in pathogenicity produced by variable strains were not measurable The dose of yeast cells that was capable of producing overwhelming infection and death in mice substantially differed from that which would produce a chronic infection Infections produced by 100 yeast like aggregates of Histoplasma capsulatum intravenously did not cause death in mice for as long as six months Intravenous dosages of 10^5 to 10^6 yeast cells killed mice on occasion Even overwhelming infections with 8×10^6 yeast cells survived for many months without evidence of infection

The disseminated and chronic lesions of histoplasmosis produced in mice by the intravenous routes as well as by intracerebral intraperitoneal and intranasal inoculation have all been comparable histopathologically When the mycelial phase of Histoplasma capsulatum is used the conversion to yeast like forms would be expected to occur within the tissues primarily infected With the

one animal there was a large necrotic area which erupted to the surface Within this lesion were noted masses of hyphae and many tuberculate chlamydospores of Histoplasma capsulatum There were no yeast phase organisms within this necrotic material No explanation can be given for this particular observation which has been unconfirmed by others

Though the intracerebral route of infection is superior in producing high mortality rates in mice (18) such an infection is completely unnatural in its acquisition However granulomatous meningitis occurs as the most constant pathologic finding following intracerebral infection and could arise from or originate the disseminated form of histoplasmosis Such lesions in mice inoculated intracerebrally with yeast like organisms have been described by Kipkie and Howell (32) but more specifically by Crayston and Salvin (19) Two days after intracerebral inoculation a predominantly lymphocytic cellular infiltration interspersed with some polymorphonuclear leukocytes was noted in the meninges Histoplasma yeast cells could already be seen within the cellular exudate The inflammatory reaction usually increased through the fourth day and was at its height by the seventh day The mononuclear infiltrates in the meninges sometimes extended along the perivascular spaces more deeply into the brain There were only lymphocytic infiltrates in some animals to extensive granulomatous inflammation with yeast-containing macrophages and a few polymorphonuclear leukocytes in others Rarely were there giant cells or epithelioid cells By the end of two weeks there was disappearance of the exudate Occasional foci of round cells were present in the brain substance After one month there were variable numbers of abscesses with necrotic centers surrounded by infiltrates of round cells and macrophages in the brain substance Many yeast cells could be found free within the necrotic material as well as within the macrophages Cellular reaction did not always accompany the presence of masses of yeast organisms

Small focal collections of lymphocytes were present in the liver two days after infection indicating early dissemination The lesions increased in number by one week and had the appearance of typical granulomas A few granulomas appeared in the spleen By one month to four months lesions became less in number and

phages containing yeast organisms surrounded the necrotic areas and some polymorphonuclear leukocytes were peripheral to this There were occasional plasma cells and polymorphonuclear and eosinophilic leukocytes scattered throughout the medulla

Lymph nodes were variably involved from a few parasitized macrophages to complete replacement of the lymph node tissue with *parasitized cells Monocytes circulating in periphera*l blood were found to contain yeast like organisms Organisms in macro phages were commonly seen in the capillaries of the glomerular tufts and in the interlobular arteries of the kidneys Though mortality might remain at a low level there appeared to be no healing what soever over an eight month period of observational cultural and pathologic study

When mice were injected via the intraperitoneal route with yeast organisms (27 28 32) the lesions were mostly confined to the liver spleen pancreas and site of inoculation Lesions were found only occasionally in the lungs heart and intestine In none of the animals thus infected were there lesions in the brain A granulo matous process with a necrotic center was extensive over the visceral surfaces Organisms were usually found to exist in the necrotic area which was surrounded by epithelioid like cells Lymphocytes were at the periphery Macrophages were filled with organisms Giant cells were rare Plasma cells and lymphocytes were found located about the periphery Focal granulomas were scattered throughout the liver and spleen It was noted that the yeast organ *isms did not always provoke a granulomatous reaction* Organisms were found to lie free in the tissues without evoking any inflamma tory response The intraperitoneal injection of the histoplasma mycelial form (6) resulted in only a very few lesions in which there were a few macrophages containing the yeast like fungus within the mesentery and the peritoneum of the abdominal cavity

There is one reported exception to finding only yeast like or ganisms in the reticulo endothelial system following dissemination of a histoplasma infection Haley (35) has made the only discovery of the mycelial sporulating stage of Histoplasma capsulatum in experimentally infected mice The animals had been infected intraperitoneally with the yeast form of the organism On the thirty second day following infection within the liver tissue of only

The many reports of naturally occurring histoplasmosis in dogs have also contributed much to the description of the patho genesis of the disseminated form of disease (38 51) The histo pathological changes in these cases of spontaneous canine histoplas mosis have also shown lesions most frequently present in lungs liver spleen bone marrow adrenals lymph nodes heart gastro intestinal tract kidneys pancreas and meninges DeMonbreun (38) being able to produce experimental histoplasmosis in puppies by feeding the fungus in milk as well as by intraperitoneal inoculation had concluded that the infection might occur via the alimentary tract Robinson and McVickar (39) in their pathologic study of 21 cases of spontaneous histoplasmosis in dogs further supported this view that the gastrointestinal tract was the usual portal of entry for the fungus They were of the opinion that the organisms after ingestion produced lesions in the intestine through which they invaded the bloodstream and were carried to the lungs and other organs

Ruhe and Cazier (55) were able to show that dogs had a con siderable natural resistance to histoplasmosis since even massive doses of the yeast organism were not uniformly fatal They pro duced experimental histoplasmosis in dogs by injecting a suspension of yeast cells into the right lung through the chest wall One of the dogs did recover from this experimental infection When this animal was sacrificed sixteen months after infection Menges *et al* (56) reported that there were no active histoplasma granulomas present histopathologically in the spleen lungs or kidneys In Para s case (45) the parasite had also been found only within the lung tissues Scattered residual vascular congestion fibrous thickening and connective tissue cells attested to the past infection Conse quently Menges (57 58) first suggested that the primary pulmonary lesion in dogs is produced by the inhalation of spores

Further substantiation of the prevalence of mild histoplasmosis in the dog and the lung as a portal of entry has been provided by Rowley *et al* (59) Emmons *et al* (60) and Schwarz *et al* (61) Dis crete intrapulmonary nodules have been studied pathologically These well circumscribed lesions were either soft and caseating or firm and calcified Peribronchial lymph nodes also contained simi lar nodules The caseating focus consisted primarily of proliferat

some livers were remarkably free from disease Only a very few mice showed activity of the lesions one to four months following infection When extensive brain abscesses were present the livers were more severely involved with granulomas scattered lympho cytes large infiltrates and many yeast cells Only occasional lesions were found in the spinal canal heart and lymphoid tissue of the gastrointestinal tract None were found in the lungs kidneys or adrenals

The production of histoplasmosis in mice via the oral route is extremely difficult to achieve as evidenced by studies in our labora tory and by Grayston *et al* (27 28)

Experimental systemic histoplasma infections in rabbits have been induced successfully (3 5 6 36 37) although susceptibility in this animal has been considered very irregular Spontaneous recovery from experimental histoplasmosis has often been observed The reactions to the intravenous and intraperitoneal injection of yeast phase Histoplasma capsulatum in rabbits is well documented (3 36 37) However only the late pathogenesis of the acute infec tion is again defined Granulomatous reaction was noted in various organs with the usual yeast engorged mononuclear macrophages

Local cellular response to intraperitoneal infection of histo plasma mycelial phase has been studied acutely and chronically in the rabbit On the second day after infection visible milk spots appeared in the omentum and grew in number and size during the first two weeks Cellularly these lesions demonstrated increasing numbers of monocytes macrophages epithelioid cells and Lang hans type giant cells with occasional foreign body giant cells The specific stimulation of the reticulo endothelial system was mani fested by this clearly monocytic involvement There was gradual regression of the inflammatory process in reverse cellular order during the third and fourth weeks By six weeks after infection the cellular reaction in the omentum had completely cleared Cellular changes in the local lymph nodes and peritoneal fluids showed monocytes epithelioid cells and giant cells as well In ani mals sacrificed after one year few areas of fibrosis and mononuclear cellular infiltrates in livers spleens and lymph nodes were noted The fungal organism could not be recognized in the tissue

Other tissues were also involved in many of the cases The adipose tissue of the bone marrow was almost completely absent The erythropoietic elements were greatly reduced Many reticulo endothelial cells containing yeast cells lymphocytes and plasma cells were present In the heart small cellular infiltrates composed of lymphocytes plasma cells and large yeast-containing mononuclear cells were located close to the blood vessels Myocardial degeneration was present in circumscribed areas Occasional neutrophils were present in the necrotic foci Within the kidneys masses of parasitized macrophages had often infiltrated the interstitial tissue and the glomerular tufts Small foci of epithelioid cells and aggregates of lymphocytes and plasma cells were contained within the cortex A few scattered glomeruli showed fibrosis Peritoneum and omentum showed infiltrates of inflammatory cells with masses of large mononuclear cells containing occasional yeast parasites Meninges were moderately infiltrated occasionally with parasitized mononuclear cells lymphocytes and plasma cells Adrenal glands were hypertrophied and contained infiltrates of macrophages which had invaded the cortex and medulla Necrosis was observed and Histoplasma capsulatum was contained in the macrophages Tonsillar tissue the pancreas and the interstitial tissue of testes contained foci of parasitized macrophages Macrophages had also infiltrated the base of several glossal ulcers

Experimental histoplasma infections in rats (61) hamsters (65) guinea pigs (66 68) chickens (69) and monkeys (4 70 71) have produced no new concept in the pathogenesis of histoplasmosis In general it can be stated that these animals have shown variable degrees of resistance to infection and consequently have been less suited for experimental study of histoplasmosis

REFERENCES

1 Day R. Experimental ocular histoplasmosis *Am J Ophth* 3 1317 1330 1949
2 Brandt F A Early tissue reactions to a South African strain of *Histoplasma capsulatum* in laboratory animals *J Path & Bact* 6 259 269 1950
3 Scheff G J and Pfeifer Scheff I M The cellular and immunological reactions in rabbits infected with *Histoplasma capsulatum* *Am Rev Tuberc* 6 374 389 1950
4 DeMonbreun W A The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum* *Am J Trop Med* 14 93 125 1934

ing epithelioid cells interspersed with a few polymorphonuclear leukocytes lymphocytes and plasma cells The regional lymph nodes contained similar large mononuclear cell tubercles with cen tral caseous necrosis Some of the lesions were highly organized and encapsulated containing central deposits of calcium Numer ous organisms within the macrophages could be demonstrated both in the pulmonary and lymph node elements of these primary lesions These lesions are considered to be the typical primary complexes which arise from inhalation of spores in nature and which simulate so well the primary lesion in man

The typical lesions of disseminated histoplasmosis have been produced in dogs by Farrell (62) after experimental infection with the yeast phase of Histoplasma capsulatum by intratracheal inocu lation No clinical disease was produced by giving dogs the same inoculum by the gastric route Although infection with the myce lial phase by either the intratracheal or intragastric routes was im possible in this study our own studies using air borne spores have resulted in successful infection of dogs resulting in both fatal dis seminated disease and uncomplicated primary infection The disseminated lesions characterized by both the naturally occurring and the experimentally induced histoplasma infection are signifi cantly the same Typical granulomas were found within the lungs liver and spleen

In lesions of the gastrointestinal tract the subserosal layer was infiltrated with lymphocytes plasma cells and numerous macro phages Infiltrates were usually heaviest in the submucosal layer and often involved Peyer s patches Necrosis and ulceration of the mucosa were frequently present in the areas of the larger infiltrates Phagocytes containing the yeast organism were present in the intes tinal wall free on the mucosal surface and sometimes about the vessels within the muscle layers Mesenteric lymph nodes showed marked proliferation of yeast engorged reticulo endothelial cells The architecture of the nodes was frequently destroyed Large foci of necrosis were present Only an occasional giant cell was seen plasma cells were in abundance Infiltration of the capsule with lymphocytes and plasma cells had occurred Areas of marked edema and fibrous proliferation were present particularly prominent about the arterial and venous channels

26 Larsh H W Cozad C C Hinton A and Furcolow M L The mouse as an aid in the isolation of *Histoplasma capsulatum* and the effect of adjuvants Pub Health Monogr No 39 1956 pp 86 92

27 Grayston J T and Altman P The pathogenesis and pathology of experimental histoplasmosis in mice (Abstract) *J Lab & Clin Med* *44* 804 805 1954

28 Grayston J T Altman P L and Cozad C C Experimental histoplasmosis in mice A preliminary report Pub Health Monogr No 39 1956 pp 99 105

29 Baum G L Adriano S M and Schwarz J Effect of cortisone on experimental histoplasmosis in mice *Am J Clin Path* *24* 903 909 1954

30 Procknow J J Page M and Looslı C G To be published

31 Hinton A Larsh H W and Silberg S L Direct exposure of mice to soils known to contain *Histoplasma capsulatum* *Proc Soc Exper Biol & Med* *94* 156 179 1957

32 Kiphie G F and Howell A Jr Histopathology of experimental histoplasmosis *A M A Arch Path* *51* 312 318 1951

33 Rowley D A and Huber M Pathogenesis of experimental histoplasmosis in mice I Measurement of infecting dosages of the yeast phase of *Histoplasma capsulatum* *J Infect Dis* *96* 174 183 1955

34 Rowley D A and Huber M Pathogenesis of experimental histoplasmosis in mice II Comparison of the intravenous and intraperitoneal routes of infection comparison of the pathogenicity of four strains of *Histoplasma capsulatum* *J Infect Dis* *97* 27 31 1955

35 Haley L D Saprophytic form of *Histoplasma capsulatum* in vivo *Yale J Biol & Med* *22* 381 383 1952

36 Salvin S B and Hottle G A Serologic studies on antigens from *Histoplasma capsulatum* Darling *J Immunol* *60* 2 66 1948

37 Hazen E L and Tahler E D Experimental histoplasmosis of skin and mucous membranes in rabbits *J Invest Dermat* *15* 205 214 1950

38 DeMonbreun W A The dog as a natural host for *Histoplasma capsulatum* *Am J Trop Med* *19* 6 587 1939

39 Robinson V and McVickar D L Pathology of spontaneous canine histoplasmosis A study of twenty one cases *Am J Vet Res* *13* 14 219 1952

40 Thuringer J M Histoplasmosis Report of its occurrence in a dog *Arch Path* *37* 140 142 1944

41 Callahan W P Jr Spontaneous histoplasmosis occurring in a dog *Am J Trop Med* *24* 363 366 1944

42 Birge R F and Riser W H Canine histoplasmosis Report of two cases *North Am Vet* *26* 281 87 1945

43 Tomlinson W J and Grocott R G Canine histoplasmosis A pathologic study of the three reported cases and the first case found in the Canal Zone *Am J Clin Path* *15* 501 507 1945

44 Seibold H R A case of histoplasmosis in a dog *J Am V M A* *109* 209 211 1946

45 Para M Histoplasmosis in Brazil *Am J Trop Med* *26* 273 9 1946

46 Harmon K S Histoplasmosis in dogs A report of two cases *J Am Vet M A* *113* 60 6 1948

5 Tager M and Liebow A A Observations on hisotplasmosis Induced infection in the mouse *Yale J Biol & Med 14* 469 488 1942
6 Parson R J Experimental histoplasmosis in mice *Arch Path 34* 229 239 1942
7 Van Pernis P A Benson M E and Holinger P H Laryngeal and systemic histoplasmosis (Darling) *Ann Int Med 18* 384 393 1943
8 Levy B M Chemotherapy of experimental histoplasmosis in white mice *Am J Trop Med 25* 241 251 1945
9 Parsons R J and Zarafonetis C J D Histoplasmosis in man Report of seven cases and a review of seventy one cases *Arch Int Med 75* 1 23 1945
10 Bunnell I L and Furcolow M L A report on ten proved cases of histoplasmosis *Pub Health Rep 63* 299 316 1948
11 Furcolow M L Further observations on histoplasmosis Mycology and bacteriology *Pub Health Rep 65* 965 994 1950
12 Emmons C W Isolation of *Histoplasma capsulatum* from soil *Pub Health Rep 64* 892 896 1949
13 Ajello L and Zeidberg L D Isolation of *Histoplasma capsulatum* and *Allescheria Boydii* from soil *Science 113* 662 663 1951
14 Grayston J T Loosli C G and Alexander E R The isolation of *Histoplasma capsulatum* from soil in an unused silo *Science 114* 323 324 1951
15 Howell A Jr Kipkie G F and Bruyere P T Studies on experimental histoplasmosis I A report on intracerebral inoculations of male dba line 1 mice *Pub Health Rep 65* 722 73 1950
16 Howell A Jr and Kipkie G F Studies on experimental histoplasmosis IV A comparison of the virulence of five strains of *Histoplasma capsulatum* by intracerebral inoculation of male dba line 1 mice *J Lab & Clin Med 36* 547 554 1950
17 Howell A Jr and Kipkie G F A comparison of the susceptibility by intracerebral inoculation of six strains of mice with male dba line 1 mice *Am J Trop Med 31* 33 41 1951
18 Howell A Jr and Kipkie G F Experimental histoplasmosis Susceptibility of male dba line 1 mice by various routes of injection *Proc Soc Exper Biol & Med 75* 121 123 1950
19 Grayston J T and Salvin S B Experimental histoplasmosis in immunized and nonimmunized mice *A M A Arch Path 61* 422 433 1956
20 Rowley D A and Huber M Growth of *Histoplasma capsulatum* in normal superinfected and immunized mice *J Immunol 77* 15 23 1956
21 Campbell C C and Saslaw S Use of mucin in experimental infections of mice with *Histoplasma capsulatum* *Proc Soc Exper Biol & Med 73* 469 472 1950
22 Strauss R E and Kligman A M The use of gastric mucin to lower resistance of laboratory animals to systemic fungus infections *J Infect Dis 88* 151 155 1951
23 Salvin S B Cultural and serologic studies on nonfatal histoplasmosis in mice hamsters and guinea pigs *J Infect Dis 94* 22 29 1954
24 Saslaw S and Schaefer J Survival of *Histoplasma capsulatum* in experimental histoplasmosis in mice *Proc Soc Exper Biol & Med 91* 412 414 1956
25 Kotcher E Robinson J W and Miller M P The isolation of *Histoplasma capsulatum* from tissues of experimentally infected mice *J Bact 62* 613 6 0 1951

°6 Larsh H W Cozad C G Hinton \ and Furcolow M L The mouse as an aid in the is lation of *Histoplasma capsulatum* and the effect of adjuvants I ub Health Monogr No 39 19 (pp 8(9

. Crayston J T and Altman P The pathogenesis and pathology of experimental histoplasmosis in mice (Abstract) *J Lab & Clin Med 44* 804 80ɔ 19ɔ4

°8 Grayston J T Altman I L an l Cz ra l C C Experimental histoplasmosis in mice \ prelim nary report I ub Health Monogr No 39 19ɔ6 pp 99 105

29 Baum C I A lriano S M an l Schwarz J Effect of cortisone on experimental histopla mosis in mice *Am J Cl n Path 4* 903 909 1ɔɔ4

30 I rocknow J J Page M and Looslı C C To be pul lished

31 Hint n A Larsh H W and Silberg S L Direct exposure of mice to soils known to contain *Histoplasma cap ulatu Proc Soc Exper B ol & Med 94* 1ɔ6 179 19ɔ7

3 Kiplıe G F and Hcwell A Jr Hı topath log) of experimental histoplasmo sis *A M A Arch Path 51* 51° 518 1951

33 Rowley D A and Huber M Pathogenes s of experimental histoplasmosis in mice I Measurement of infecting dosages of the yeast phase of *Histoplasma capsı latum J Infect Dis 96* 174 183 19

34 Rowley D A and Huber M I athogenesis of experimental histoplasmosis in mice II Comparison of the intraven us and intraperitoneal routes of infec tion comparison of the pathogenicity of four strains of *Histoplasma capsula tum J Infect Dis* 9 27 31 19ɔɔ

35 Haley L D Saprophytic form of *H st plasma capsulatu ı n v vo Yale J B ol & Med 24* 381 383 19

36 Salvin S B and Hottle G A Serolc gic stı lies on antigens from *Histoplasma c psulatum* Darling *J Imm ınol 60* 7-6(1918

37 Hazen E L and Tahler E D Experimental hı toplasmosis of skin and mucous membranes in rabl ıts *J Inv st De mat 15* °0ɔ °14 19 0

38 DeMonbreun W A The dog as a natural host for *Histoplasma capsulatum Am J Trop Med 19* 6 587 1939

39 Rob nson \ and McVıckar D L Pathology of spontaneous canine histoplas mosis A stu ly of twenty oı e ca es *Am J Vet Res 13* 214 219 195°

40 Thuringer J M Hist pl smo ı Rep rt of its occurrence in a dog *Arch Path 37* 140 14° 1944

41 Callahan W I Jr Spontaneot s h toplasmosis occurring in a dog *Am J T op Med 24* 3(3 3((1944

4° Burge R F and R ser W H Canine histoplasmosis Report of two cases *No th Am Vet* 6 281 87 1945

43 Toml nson W J and Grocott R G Canine l stopla mosis A pathologic study of the three repo ted cases and the first case found in the Canal Zone *Am J Clin Path 15* 501 507 1945

44 Seibold H R A case of histoplasmosis in a dog *J Am Vet M 4 109* 209 11 1946

45 P ra M Histoplasmosis n Brazil *Am J Trop Med* 6 °73 9 1946

46 Harr on K S Histoplasmosis in dogs A report of two cases *J Am Vet M A 133* 60 6° 1948

47 Cordy D R Histoplasmosis in a dog Cornell Vet 39 339 343 1949

48 Mosier J E Barner R D and Davis J C Histoplasmosis in dogs J Am Vet M A 116 1 8 131 19 0

49 Oxebell J W Histoplasma capsulatum infection in a dog North Am Vet 31 40 1 19 0

50 Bentinck Smith J Kennedy E C and Saunders L Z Histoplasmosis in a dog Cornell Vet 42 [illegible] 19

51 Cole C R Chamberlain D M and Prior J A Incidence symptomatology and diagnosis of canine histoplasmosis Am Vet M A Proc Book 1951 pp 1/9 181

52 Cole C R Farrell R L Chamberlain D M Prior J A and Saslaw S Histoplasmosis in animals J Am Vet M A [illegible] 1953

53 Eby C H and Ringen L M Canine histoplasmosis in the Pacific Northwest J Am Vet M A 1 7 250 2 1 19

54 Fish N A Schroder J D and Fischer J B A laboratory report on a case of canine histoplasmosis in Ontario Canad M A J [illegible] 3 19 6

55 Rube J S and Carter I D A review of histoplasmosis J A Vet M A 115 47 0 1949

56 Menges R W Furcolow M L and Rube J S Experimental histoplasmosis in a dog A nonfatal case Pul Health Rep 65 6 8 631 19 0

57 Menges R W Canine histoplasmosis J Am Vet M A 119 411 415 1951

58 Menges R W McClellan J T and Ausherman R J Canine histoplasmosis and blastomycosis in Lexington Kentucky J Am Vet M A [illegible] 907 19 4

59 Rowley D A Haberman R T and Emmons C W Histoplasmosis pathologic studies of fifty cats and fifty dogs from Loudoun County Virginia J Infect Dis 95 98 108 1954

60 Emmons C W Rowley D A Olson B J Mattern C F T Bell J A Powell E and Marcey F A Histoplasmosis Proved occurrence of inapparent infection in dogs cats and other animals Am J Hyg 61 40 44 1955

61 Schwarz J and Bingham F L The pathogenesis of canine histoplasmosis J Am Vet M A 128 611 613 1956

62 Farrell R L Cole C R Prior J A and Saslaw S Experimental histoplasmosis I Methods for production of histoplasmosis in dogs Proc Soc Exper Biol & Med 84 51 54 1953

63 Cole C R Experimentally induced histoplasmosis in a dog J Am Vet M A 127 596 598 1955

64 Middleton J G McVickar D L and Peterson J C Experimental histoplasmosis in the white rat Proc Soc Exper Biol & Med 75 161 166 1950

65 Drouhet E and Segretain G Histoplasmose experimentale chez le hamster doré Ann Inst Pasteur 83 381 383 19.9

66 Reid J D Scherer J H Herbut P A and Irving H Systemic histoplasmosis Systemic histoplasmosis diagnosed before death and produced experimentally in guinea pigs J Lab & Clin Med 27 419-434 1942

67 H well A Jr Isolat on of pathogenic fungi from experimentally inoculated guinea pi *Pul Health Rep* 63 60 616 1948

68 Allen R M Experimental histoplasmosis portal of entry of the fungus *Am J Trop Med* 8 83 861 1948

69 Menges R W and Habermann R T Experimental avian histoplasmosis *Am J Vet Res* 16 314 320 1955

70 Wright R B and Hacht L F W Histoplasmosis of Darling report of a case *Ann Int Med* 15 309 313 1941

71 Hill C A and Marcus S Challenge of *Macacus irus* with *Histoplasma capsulatum* *Am Rev Tuberc* 75 819 8 1 19

THE PATHOGENESIS OF HISTOPLASMOSIS IN THE HUMAN BODY

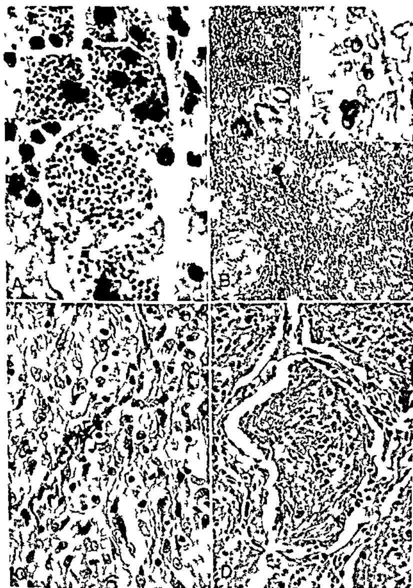
HENRY C SWEANY

The beginning and course of histoplasmosis in man still has many unsolved aspects The progressive and fulminating type where there is a rapid sequence of progression from one organ to another with the finding of parasites in all the organs and even in the blood stream was first observed and described by Darling (1) and later by several others The more chronic forms and especially the types having encapsulated caseous or calcified lesions still offer some problems for solution The various known courses and possibilities will be reviewed

The experimental work of Procknow (2) on the course of the spores of H capsulatum in the mouse is perhaps a good indication in favor of a similar course in man Since this experiment cannot be repeated in man we must make an assumption with some justification that after the spores are inhaled they go through a similar cycle in man to what has been shown in mice Furthermore we may assume that on inhaling many to hundreds of spores as is frequently observed the same process is repeated over and over again at each small focus where the spores are located in the alveoli or in the smaller bronchioles

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Fig 1 (A) Adrenal gland demonstrating histiocytes packed with Histoplasma capsulatum H & E x820 AFIP Acc 61428 (B) Abdominal lymph node with epithelioid-cell granulomas resembling lesions of Boeck s sarcoid H & E x113 AFIP Acc 611205 Insert (right upper) Gridley fungus-stain demonstrating Histoplasma organisms within an epithelioid-cell granuloma of this lymph node x1250 (C) Subcutaneous nodule in which the reaction resembled fibrosing granulation tissue Only a few Histoplasma cells were seen H & E x445 AFIP Acc 89775 (D) Lung in which the reaction was that of organizing pneumonitis H & E x267 AFIP Acc 206008 With special stains many Histoplasma organisms were seen (Used by permission of the *Am J Clin Path* the author Chapman H Binford and the Armed Forces Institute of Pathology Washington D C.)


A
B
D

After these preliminary assumptions are made, there are still unknowns for the disease process has many variations. At one time it seemed to be a rather *simple process extending from the primary focus to the adjoining lymphatics*, lymph nodes, the blood stream and then to the various organs throughout the body. In the progression the yeast bodies are found in great profusion in the macrophages and caseous foci. Since these patients usually do not survive but a few months, large numbers of parasites can usually be found in all the organs. But the rapidly progressive infections are now known to be rare compared to the more slowly progressive types.

Recently it has been observed that single lesions sometimes with one or several satellites may be the most common first localization. The course seems to parallel that of primary tuberculosis in its principal aspects with calcification and apparent healing. Straub and Schwarz (3) and Schwarz (4) independently in this monograph *have indicated strongly that the course is in many respects similar* except in the causative agents. The parasites which are not genetically, morphologically, or chemically like tubercle bacilli do grow at a comparable *slow rate of speed* and perhaps are met by similar body defences as they advance through the body. There has been such an extensive accumulation of facts regarding the course of tuberculosis in the body, including the primary, postprimary and true re-infection, that it would seem that the principal courses and actions in the body are now established facts. The temptation is great, therefore, to apply everything that is known concerning tuberculosis to histoplasmosis since so many features are similar and the evolution of the lesions follow along similar lines.

It would be risky, however, to assume too much similarity since there has not been time enough or sufficient material of histoplasmosis from which to draw definite conclusions. Already there are observations on histoplasmosis that do not follow those that have been described for tuberculosis. For example, there are only a few instances in the literature where primary tuberculosis begins as a shower of lung lesions with a corresponding involvement of the lymph nodes. Owing to its nature, histoplasmosis is frequently, if not predominantly, seeded in that manner.

Another difference that is borne out by roentgenological and pathological evidence is the greater tendency of lesions of histo-

plasmosis to take up calcium (5 6) While both diseases calcify the difference is too regular to disregard in both the primary lesions and also in the large coin or spherical lesions The reasons for the greater tendency for the lesions of histoplasmosis to calcify is still obscure Perhaps there is greater local activity than in tuberculosis more enzyme action and more proteolysis creating more free ions for union with the basic calcium which seeps in from the blood stream

The lymphatics of the peribronchial regions and the mediastinum are more prone to develop tumor like masses simulating cancer or resulting in pressures that have been found to lead to bronchiectasis (6) and even blood inflow stasis to the heart by constriction of the vena cava From the limited observations that have been described so far regarding this phase of histoplasmosis it would seem that they will outnumber any similar type of formation that has been described for tuberculosis

Whatever the cause may be there has appeared a difference that has become obvious to all those who have studied the two diseases in the greater incidence of multiple lesions in the spleen in histoplasmosis Schwarz (7) feels that any case having more than four to five calcified lesions in the spleen may be considered as histoplasmosis In most places today that is a pretty good rule but it must be remembered that the observations on histoplasmosis have been made after the advent of drug therapy for tuberculosis and at a time when huge doses of tubercle bacilli have been reduced by public health methods

Although the spleen is involved more in histoplasmosis tuberculosis may also cause disseminated lesions (See Fig 1(B)) In the older literature multiple calcifications as well as caseous tuberculous lesions of the spleen (8) have been reported In my own studies (9) I have found several cases mostly in young negroes where multiple caseous lesions have been found in tuberculosis with some undergoing calcification At the time of these observations tubercle bacilli were found present in all but two of these cases It must be admitted that no stains were made at that time for the parasites of histoplasmosis but since the patients were out and out tuberculous in every way and since the findings were observed in a region where histoplasmosis was not common it seemed logical to consider

them tuberculous. We can't draw too sharp a line therefore regarding the number of calcifications caused by either disease since both diseases are similar in their progression throughout the body and the nature of the lesion produced. The cause of the difference partly at least was that most patients died of tuberculosis before the lesions in the spleen had time to calcify. The general trend in tuberculosis is for the disease to terminate early when many caseous foci appear in the spleen whereas in histoplasmosis the process is relatively much more benign and more lesions persist and become calcific.

The general rule at this time and in the endemic areas particularly is that many dense calcific lesions in the spleen may be considered as histoplasmosis.

As the progression of the disease continues throughout the body there is a tendency for the parasites to locate in certain other organs beyond the lymphatics (in addition to the spleen) notably the adrenals, bone marrow and liver. In disseminated tuberculosis the same organs are involved but from the evidence to date there appears a far greater tendency for histoplasmosis to involve the adrenals than does tuberculosis. Binford (10) found 21 out of 22 fatal cases with involvement of the adrenals. Rarely does tuberculosis strike the adrenals heavily at its first onslaught but histoplasmosis attacks them commonly in the progressive and overwhelming types of the disease. The tuberculous infection of the adrenals usually do not produce symptoms until years after the first infection when an Addisonian syndrome may appear.

From the histories of many chronic cases in our studies the disease process seems to follow a definite pattern. First is in cases that have had strong possibilities of exposure to dust that may be suspected of bearing spores. Second the patients are generally in the latter half of life and undergoing early deterioration. Most of our cases have been found in farmers between 50 and 75 years of age. That is the resistance to all infections has been lowered and either a new seeding of spores has taken place or there has been an exacerbation of an old lesion. Both of these courses have been found in cases coming under our observation. Many have shown recent infiltrative lesions with or without lymph node complexes that seem to point to a recent infection. Others have shown a definite

exacerbation in and around caseous and calcified lesions where there had been an early infection and for various reasons the parasites were re awakened and set up a new and active disease process

The question of a re infection on an old entirely healed infection is one that cannot be answered at this time for it is not known when the first infection is healed if it is ever really healed with all the parasites dead The problem of the life of the parasites in old lesions is therefore one that is difficult to determine and certainly difficult to resolve by experimental means It can best be done by long observation of a great many cases where clinical x ray and laboratory studies have been carried out In a few cases in our work this has been accomplished but up to this time we have been content to demonstrate parasites in old lesions rather than to try to prove their viability From the appearances of the yeast in old lesions most of them seem to appear no different than other known living yeast Yet in a few attempts to grow them our results have been entirely negative Puckett (9) was able to obtain only one positive culture out of 22 cases of round lesions or as they have been called coin lesions It is doubtful if these results represent the true situation for we have not been able to obtain growth in any encapsulated lesion although several have been recent and progressive with abundant parasites present as shown by the G M S stain Methods are still inadequate therefore to grow the forms that we are able to identify not to mention the possibility of any unrecognizable forms

It may be well to state at this point that less than half of the open active cases have produced growths on medium on the first few attempts made The remainder have required numerous culture plates and then perhaps only one or two colonies appeared Many times the colonies are overgrown by contaminating yeasts especially by Candida and bacteria

There are some observations that may be worthwhile in indicating possible viability in old lesions One is the presence of buds on the yeast bodies Budding is evidence of recent growth activity and many old lesions have shown budding and short elongated bodies that may be considered abortive mycelia Several ossified lesions have shown budding in the yeast bodies The other questionable indication is the presence of good birefringence on polar

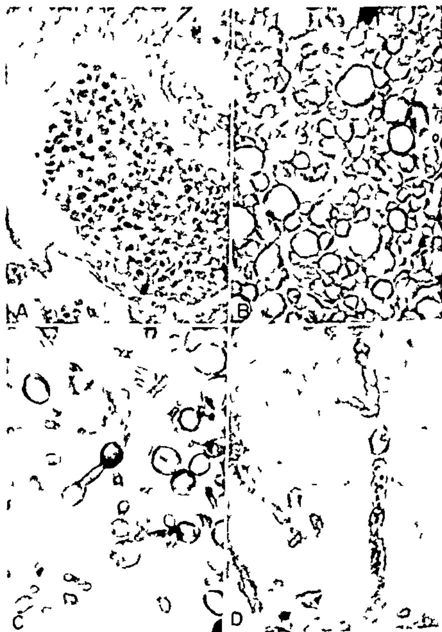


Fig 21 Section of thrombus aortic valve Photomicrographs were all made from sections taken from the same block and represent areas only a few high power microscopic field apart AFIP Acc 204556 (No culture for fungi →

iscopy In some very old lesions the parasites have been merely ghosts with poor or no birefringence at all This feature is at no time reliable for it is recognized that polysaccharides may remain long after the parasites are dead The question of the length of time parasites survive in encapsulated lesions therefore is one that has caused much concern and is far from settled

In studying a large number of lesions there is commonly a great variation in the size or shape of the parasite Some of them appeared to be rapidly growing by budding Others were stationary and others undergoing various stages of degeneration down to mere ghosts of the original There is no doubt that the parasites are vulnerable and are destroyed in some lesions or seem to lose their usual morphology Sizes vary from one micron up to five microns

A few words should be said about the variation in size of the parasite in different organs of the body and in the various types of disease In the fulminating and fatal forms there is generally a continued progression in the reticulo-endothelial cells of the round or pear shaped yeast bodies of average size (3 5 microns) As caseous centers develop there is a marked destruction of the parasites leaving ghosts and the granular debris mentioned before Under certain conditions as shown by Binford (10) the parasites may become greatly enlarged up to 15 20 microns in diameter and sometimes there is production of abortive and even some genuine hyphae The hyphae may lengthen into many joints and actual threads of some length What bearing these changes have on the development of lesions is not known It may be that the formation of hyphae is the result of a certain type of lesion rather than a typical process

An observation that has been increasingly common is the formation of one two or even several granular residues in the ends

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Case originally diagnosed moniliasis Lesions in adrenal gland typical for histoplasmosis) (A) Viable tissue of heart valve Histoplasma of normal size which stained well with hematoxylin and eosin x1250 (B) A field from within thrombus but adjacent to A Large forms almost invisible with hematoxylin and eosin stain but well stained with Gridley fungus stain x1250 (C) In thrombus adjacent to B but nearer inner surface of thrombus Large forms with hypae Gridley fungus stain x1250 (D) Inner surface of thrombus Septate hyphae Gridley fungus stain x1250 (Used by permission of *Am J Clin Path* the author Chapman H Binford and the Armed Forces Institute of Pathology Washington D C)

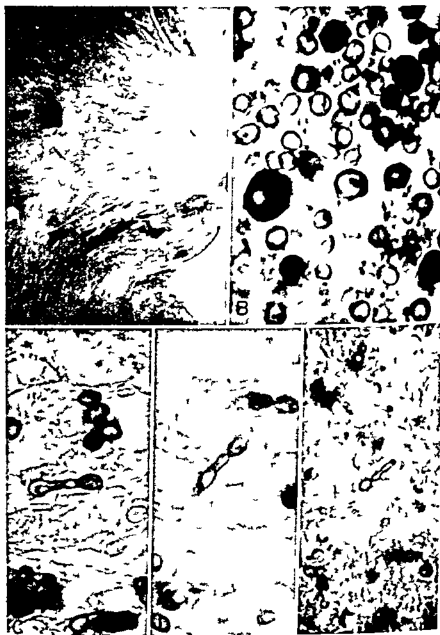


Fig 2b (A) Necrotizing renal papillitis caused by Histoplasma capsulatum H & E x6 AFIP Acc 325234 (B) Duplicate section of A The large forms ⟹

and walls of the old parasites Darling s original work has sketched the inclusions in the yeast quite clearly At first these small forms were passed by but after seeing them in increasing numbers and also recalling the times we have seen microspores free or in a matrix with other typical yeast and abortive buds left in old ghosts it occurred to us that these bodies deserved study and more serious consideration The reference of Binford (10) to microspores as small as one micron in size and more especially the fine detailed description of Pine (11) who observed inclusion bodies in H capsulatum seem to indicate that there may be significance to these small forms and there may be more yet to the evolution of the parasites than is known at present While no budding of the smallest forms has been demonstrated typical birefringence has been observed on the forms less than 0 5 of a micron and transition forms have been seen up to 2 or more microns In many instances these forms arose in the ghosts of yeast bodies On observing a great many of these small granules they seemed to represent a budding process that was interrupted by a too rapid destruction of the yeast leaving the bud as an abortive granule just protruding from the tip of the yeast or actually trapped in or just beneath the wall If any proof is ever found to the theory of origin of microspores it may help to clear up some of the underground phases of histoplasmosis where the regular yeasts cannot be found

Following a discussion of microspores it is fitting now to discuss the development of the various types of chronic lesions After the first appearance of the parasites in the chronic forms there seems to be many courses taken by the disease process The first is the infiltrative lesion that resembles chronic inflammatory infiltrates of tuberculosis abscess or even cancer The parasites appear to be phagocytized by the macrophages and in the early phases may be stained by PAS and the Gridley stain They soon disappear from

were present only at the tip of the necrotic papilla Periodic acid Schiff method with light green counterstain x1250 (C and D) Necrotic adrenal gland in a case of histoplasmosis Short hyphae and moderately enlarged forms Gridley fungus-stain x1250 AFIP Acc 64868 (E) Necrotic renal medulla in histoplasmosis Note short hypha Gridley fungus-stain x1250 AFIP Acc 579628 (Used by permission of the *Am J Clin Path* the author Chapman H Binford and the Armed Forces Institute of Pathology Washington D C)

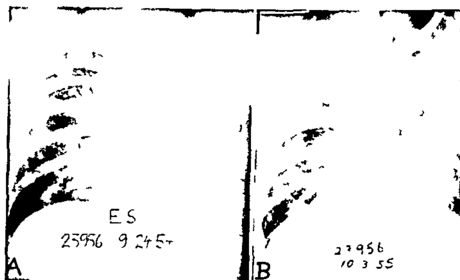


Fig 31 (A) Roentgenogram of E S B15 No 23 956 taken on September 24 1954 showing a consolidated left lung (B) The same as Figure 31 (A) taken on October 3 1955 with a double exposure to show the nature of the lesion on the left This patient was always positive for a f b and turned negative on antituberculosis drugs (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*

view however and the G M S stain reveals only small black dots that cannot be distinguished from dark staining artefacts Yet they seem to be present within the macrophages and may be remnants of the parasites So far as we know now however the parasites *to all intents and purposes have been destroyed in the macrophages* After the caseous mass becomes encapsulated months and years later however typical yeast bodies almost always appear in the caseous centers This has been observed so often that it seems that the parasites have arisen from some occult source Sometimes small granules may be seen in the caseous centers that have bluish green staining matrix associated with definite yeast bodies Later on typical yeast bodies may develop in these small colonies It is possible for a few typical parasites to have been overlooked but in most of the chronic lesions the yeast forms are so numerous that some other origin must be suspected Since the yeast bodies are non motile and are placed at regular intervals nearly always in large colonies it seems highly



Fig 3b *Left* (A) A low power view of a section of a dense parenchymal calcification taken from the resected left lung of patient shown in Figures 3a (A) and 3a (B) Two areas are outlined in the center of the lesion one by dots at a and another by black line at b H & E x15 (B) A squared off area of Figure 3b (A) showing bony character of lesion H & E x 20 *Right* (A) A microscopic view of region marked at a in Figure 3a (A) showing numerous typical yeast bodies G M S stain x1066 (B) Polariscopic view of (A) x1066 (C) A view of field marked off at b in Figure 3a (B) Note the numerous small dark staining bodies that may be developing into yeast bodies G M S stain x1066 (D) Another view of the field of b in Figure 3a (A) G M S stain x1066 (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*



probable that they have arisen from some obscure source Further more there are transition forms present that seem to bridge the gap to more typical yeast bodies These possible changes however are only speculative and at present are to be considered only as possibilities

If and when the lesion sloughs into a cavity cultures usually become positive and the parasites appear in the cavity walls especially in the caseous centers of the walls The peripheral macrophages usually do not reveal any parasites at this stage of development Whether the parasites were ever present in them is only conjectural at present

In the encapsulated lesions the parasites are usually found in great numbers far in the interior of the encapsulated mass and almost entirely in centers where recent caseation has developed Even in old ossified lesions small pockets of parasites may be found in the centers of the lesions Some appear fresh and vigorous others may be very small and in a matrix while others are poorly stained and ghost like As already stated the time these bodies may survive is still one of the least understood problems of histoplasmosis It seems safe to assume that so long as budding is present life was present at least a short time before

As the disease becomes chronic and fibroid there is a tendency for healing to take place in the walls of the cavities but the process seems to extend into the tissues and the cavity walls become thicker and usually hyalinized but nearly always the surfaces become slick and cyst like At the same time the disease is progressing slowly into the tissues outside of the wall of the cavity

The round lesion as first described by Puckett (12) may keep enlarging when it is difficult to find any parasites either by culture or staining In some lesions numerous sections have failed to reveal parasites while others only show them in the early pockets of casea

Fig 4 (The lesion from which these are taken are shown in the x ray in Chapter 2, Figures 1 and 2 by Dr John Polk) (A) (*Upper*) B 30 L B 26 578 Showing macrophages in the periphery of the lesion with what might be parasites PAS stain x1066 (B) (*Center*) Same G M S stain showing coal pigment and small black bodies that may be nuclei of yeast (C) (*Lower*) Same as (B)

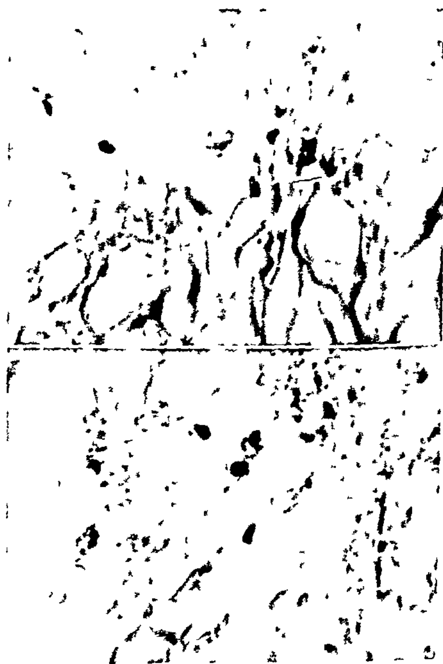




Fig 6 Typical round lesion (coin lesion) of proven Histoplasmomas at (A) *(left)* and proven tuberculomas at (B) *(right)* (Reproduced from Myers J A Editor *Diseases of the Chest Including the Heart* (Springfield Thomas 1959) by permission of the Editor and Publisher This and other illustrations by the author originally appeared in an article by Sweany C relick Coller and Jones *Dis Chest* 34 6 1958)

tion that develop late in the process and well toward the center of the lesions As mentioned before positive cultures on encapsulated lesions is rarely ever found yet apparently viable forms exist

In our work we noted two types of round lesions one a gradually expanding lesion that builds up like the winding of a ball of twine another where there is an encapsulated infiltrate varying in size from a few millimeters to 4 or more centimeters

One of the greatest enigmas in the lesions of histoplasmosis is the formation of chronic pneumonitis There is a type that may be called a chronic pneumonia with organizing fibrinous exudate con

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Fig 5 (Same as Figure 4—Margins of caseous area) (A) *(Upper)* B 30 No 26 328 A high power view of edge of caseation showing fibrosis below and caseation above with the appearance of bean shaped bodies that may be parasites starting to grow G M S x1066 (B) *(Lower)* Same except the development is more advanced

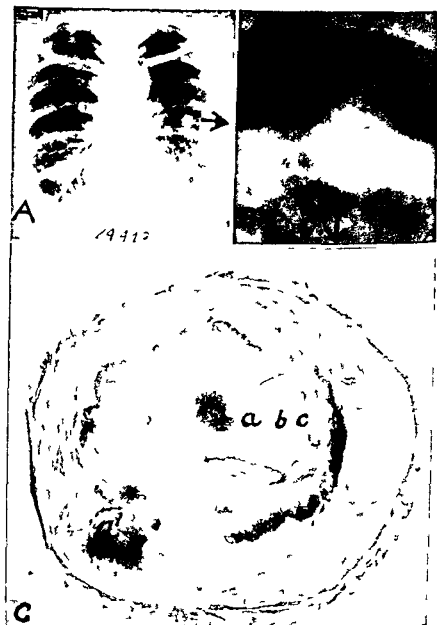


Fig 7 Taken from Pathologic findings in benign pulmonary histoplasmosis by Sweany *et al* (*Dis Chest 34* 6 1958) (A) A reduced roentgenogram of E L S A 15 No 24 493 Showing a spherical lesion in the left midfield with

taining a few macrophages and the other type a chronic granuloma that sometimes can't be distinguished from sarcoidosis Both of these types have been well described by Binford and others Parasites are rarely if ever found in either type and in the cases I have studied no granules have been observed

The evolution of ordinary re infection type of lesion is probably much like the evolution of a similar infiltrative tuberculosis process where there is usually no involvement of the lymph nodes The lesion becomes caseous in the center and after a time shows a tendency to form stellate crevasses in the center that soon find their way into the adjoining bronchi after which there is a gradual shelling-out of the cavity New foci form more toward the base of the lung as in tuberculosis and the same evolution takes place all over again There is a tendency of these cavities to slough out the caseous material which is free of polymorphs and leave behind a slick walled cavity This is a departure from the progress in untreated tuberculosis which keeps a pyogenic membrane made up largely of polymorphs with a smaller number of monocytes and lymphocytes

A perplexing problem is presented in round lesions that are typical histoplasmomas with onion ring formations but wherein no foci of caseation nor yeast bodies were found Serial sections may have found both caseous foci and parasites since only about 0 1% of the lesion is represented on one 6 micron section

Alternative views are that there are no living parasites remaining or that there is a silent phase that has not yet begun to metabolize and produce caseation with typical yeast bodies

Irrespective of the ultimate cause and result an earnest entreaty to pathologists is to search diligently for caseous—preferably encapsulated caseous—foci where yeasts are found

In addition to the various unknowns already discussed so much is yet to be learned about the course of chronic histoplasmosis in the body that a few questions may be raised at this time We know

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pleura at the left base and bronchiectasis in the right middle lobe (B) An enlargement of the spherical lesion of (A) (C) A low power section of spherical lesion Note the two centers of dense caseation and calcification outlined by small dots at a and a and the partial rings of less marked calcification (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

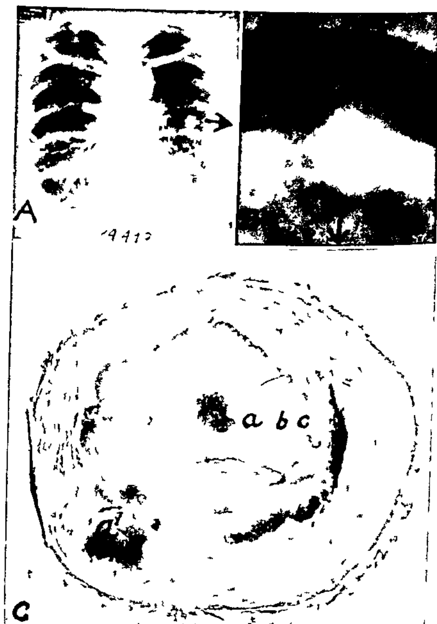


Fig 7 Taken from Pathologic findings in benign pulmonary histoplasmosis by Sweany *et al* (*Dis Chest 34* 6 1958) (A) A reduced roentgenogram of E. L. S A 15 No 21 493 Showing a spherical lesion in the left midfield with



Fig. 9 (A) A microscopic field of a G. M. S. stained section in the dense central calcification marked "a" in Figure 7(C) showing innumerable yeast bodies. x1133. (B) A polariscopic view of same showing Maltese crosses. x1133. (C) A microscopic field of stained section extending outward into the zone marked "b" in Figure 7(C). G. M. S. stain x1133. (D) A microscopic field of stained section still further out toward the periphery. (E) Similar to (D). (Used by permission of Dr. J. A. Myers, Editor, and *Diseases of the Chest*.)

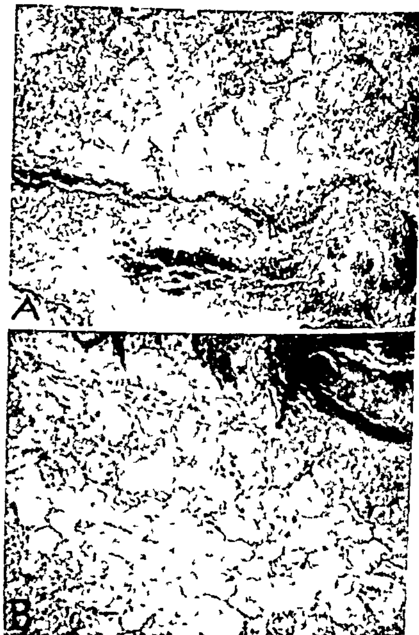


Fig 8 (A) A microphotograph of the central area of Figure 7(C) (the blood vessel marks the general location) Note the outlines of the alveoli caused by the calcium deposits in the alveolar wall and possibly to a calcium salt of nucleic acid in the disintegrated nuclei of the alveolar walls x48 Von Kossa stain (B) Another view similar to that of (A) (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

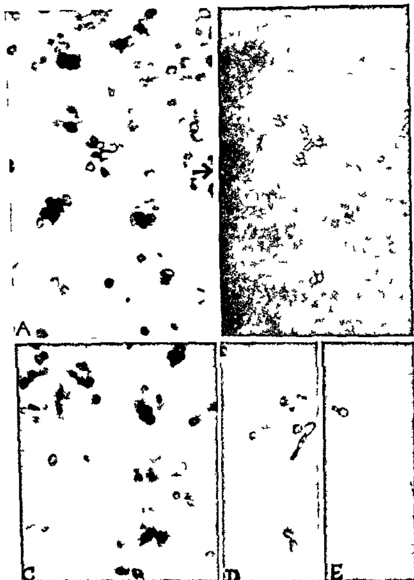


Fig. 9 (A) A microscopic field of a G. M. S. stained section in the dense central calcification marked at "a" in Figure 7(C) showing innumerable yeast bodies. x1133. (B) A polariscopic view of same showing "Maltese crosses." x1133. (C) A microscopic field of stained section extending outward into the zone marked "b" in Figure 7(C). G. M. S. stain x1133. (D) A microscopic field of stained section still further out toward the periphery. (E) Similar to (D).
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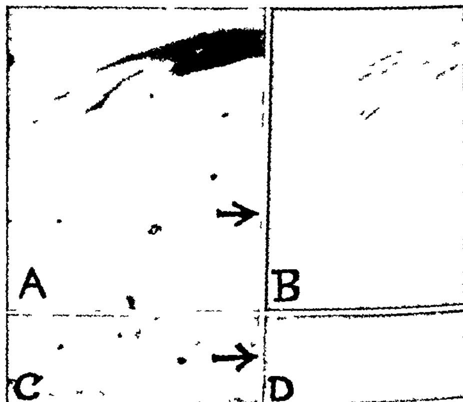


Fig 10 (A) A zone marked by c in Figure 7(C) where there is still fibrous tissue The dark staining bodies are smaller and assume a round granular appearance Most show no birefringence but one in upper left is typical G M S stain x 1133 (B) Polariscopic view of (A) Only 2 or 3 show a faint birefringence G M S stain x1133 (C) Still further out in the fibrous tissue G M S stain x1133 (D) Polariscopic view of (C) Only one shows typical birefringence Others only show points of light They may be artefacts x1133 (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

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Fig 11 (A) A stained section of a spherical lesion of M S 139 S203, 53 showing the ring formation from the center outward Note the heavy callous on one side This heavy button like plaque on one side is present on several spherical lesions and its cause is not understood H & E x4 5 (B) A higher magnification of area marked off in (A) The calcification does not follow the fibrils but is more related to distance from the center H & E x20 (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

that in tuberculosis where there has been a small healed primary infection that a re-infection complex may form. Do all calcifications in the parenchyma and lymph nodes in histoplasmosis represent a primary lesion or may it not be a re-infection complex as sometimes occurs in tuberculosis? May not heavily infected people who expectorate large numbers of parasites in the sputum pass the infection to others as is done in tuberculosis? It seems possible but no case has yet been found. Are there any other portals of entry than in the lungs such as the tonsils, gastro-intestinal tract, or the skin? Outside of penile lesions reported by Curtis and Harrell (13), Curtis and Cawley (13a), and Van Pernis and associates (14) laryngeal infection, nothing definite yet has been reported.

In conclusion, it may be stated that what once looked like a relatively simple process has turned out to have many unknown phases and obscure facets of the disease histoplasmosis, especially in its retrogression, healing, and exacerbation. The relationships of the known forms of the parasite is not the process it appeared to be when only the fulminating types were known. Now there has appeared many new aspects and many gaps in our knowledge of the host parasite relationship. Much depends on a more thorough knowledge of what happens to the parasite in different situations and in the many chronic phases of the disease. Pathogenesis of the disease in the human body is therefore only partly understood and the unknowns can only be resolved by a more intense program of study.

REFERENCES

1. Darling, S. T.: A protozoan general infection producing pseudotubercles in the lungs and focal necroses in the liver, spleen and lymph nodes. *J.A.M.A.* 46: 1283, 1906.
2. Procknow, J. J.: This monograph.
3. Straub, M. and Schwarz, J.: Healed primary complex in histoplasmosis. *Am. J. Clin. Path.* 25: [illegible], 195[illegible].
4. Schwarz, J.: This monograph.
5. Davis, F. W., Peabody, J. W., Jr. and Katz, S. L.: Solitary pulmonary nodule: ten year study based on 21[illegible] cases. *J. Thoracic Surg.* 3[illegible]: [illegible], 19[illegible].
6. Sweany, H. C., Gorelick, D., Cutler, F. C. and Jones, J. L.: Pathology of findings in benign pulmonary histoplasmosis. *Dis. Chest* 34: 119-13[illegible] and 2[illegible]-2[illegible]5, 19[illegible]8.
7. Schwarz, J., Silverman, F. N., Adriano, S. M., Straub, M. and Levine, S.: The relation of splenic calcification to histoplasmosis. *New England J. Med.* 25[illegible]: 887-891, 195[illegible].

8 Wintermitz M C Tubercul sis of the pleen *4 cl I it M d* 9 680-697 1919

9 Sweany H C O the nature of calcif l lesions with pecial reference to those in the spleen *Am J Poe tg n I 44* 909 1941

10 Binford C H H stopla m 1 s e rea ti n and i orp l l c variations of the fungi s *J Clin I tl* 5 2 1

11 line I This monograph

1 Puckett T F Puln nary H topla m sis—a tu l of cases with i lentif cation of H cap ulat n the resected ti sues *4 Rev T ibere 6* 4 3-4 6 19 3

13 Curtis and Ha rel C ted in Wilson J W *Cl n al a d Im lo cal Aspects of Fu gi s D ea e* Spr gfield Il mas 19

13a Curtis A C a l Cawle F I C nital hi t plasn si *J U r I 5* 194

14 Van Pernis I A Bens M E and H lin er P H Specific cutaneous reac t on th hi topl asm s pulmon ry report f a tl er case *J A M A 11* 436 43 1941

THE PRIMARY LESION IN HISTOPLASMOSIS

Jan Schwarz

INTRODUCTION

The primary lesion in histoplasmosis has been recognized only recently even though its presence on x ray and at autopsy must have been noted by many examiners in the form of caseated or calcified foci Such lesions were interpreted arbitrarily until lately as tuberculous After it was shown that many persons with calcified intrathoracic lesions failed to react to tuberculin (1 8 11) it was concluded that the cause probably was not tuberculosis No positive answer about the anatomical findings was given until the work of Christie (3) Schulz (14 15) and Straub (18) Schulz (14 15) has described in 4 autopsies the presence of active primary complexes documented with beautiful gross pictures and demonstration of the organism Straub (18) specifically has established the constant development of a primary complex after infection with Histoplasma capsulatum He demonstrated this entity in 67% of non selected routine autopsies in the endemic area At the point of the primary infection a pneumonitis develops which in the majority of cases conduces to necrosis and caseation The early lesions which have been observed are circumscribed and very similar to the primary lesions in tuberculosis They are located subpleurally and are discrete but they frequently get much larger than the corresponding tuberculous lesion The organism Histoplasma capsulatum can be demonstrated throughout the lesion when active and in the center of the lesion even after many years The pleural reaction is as a rule minimal and may consist of slight fibrinous pleurisy or of an outpouring of a small number of round cells much less frequently polymorpho nuclear leukocytes Small satellite nodules often develop in the immediate vicinity of the primary focus and later may

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be incorporated into the caseation of the main focus How much and how often the incorporation of such collateral foci contributes to the growth of the primary focus is hard to assess

Parrot (12) has stated in 1876 that the lymph nodes of the



Fig 1 Active primary complex in histoplasmosis of 15 months old girl Large caseous primary focus subpleural in lower third of lower lobe Caseation of regional lymph nodes which are markedly enlarged Disseminated histoplasmosis with innumerable miliary and somewhat larger foci in the pulmonary parenchyma (Courtesy of Dr Dale M Schulz from *Am J Clin Path* 24 1196 1954)



Fig 2 M S Primary active focus of histoplasmosis Thin and incomplete fibrous wall surrounding the focus Lymphangitic streaks and perifocal inflammation are present Formation of small secondary foci outside the wall are indications for the mechanism of potential increase in size of the primary lesion (Van Gieson Elastica—27x)

pulmonary hilus always reflect like a mirror the pathology developing in the parenchyma of the lungs Il n est pas a cette periode de la vie d affection pulmonaire qui ne se reflete d une maniere tres-nette sur les ganglions bronchiques ils sont comme le miroir des poumons et reciproquement il n y a pas d adenopathie bronchique qui n ait une origine pulmonaire This *law of Parrot* applies to histoplasmosis as it does to most other granulomatous diseases *

The formation of a primary complex (consisting of the parenchymatous focus and satellite lymph nodes) is most conclusively seen in tuberculosis syphilis sporotrichosis coccidioidomycosis rickettsial diseases etc If this principle would be more widely known the many case reports of primary laryngeal cutaneous oral anal etc fungus infections would have never been written They bespeak rather eloquently the ignorance of the writer about this principle the law of Parrot

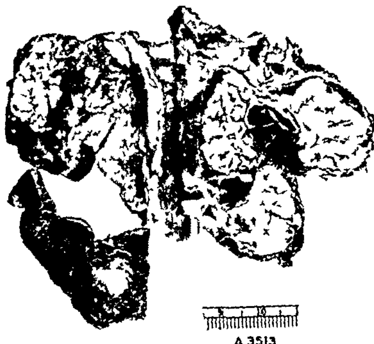


Fig 3 A 3513 Large calcified (arrested) primary focus with relatively soft rim and rock like centre in the lung Huge satellite lymph nodes with masses of stippled calcification in peribronchial location The medial half of the lymph node at left has been removed to permit visualization of bronchial lumen Multiple splenic calcifications were found in this person

The lymph nodes form an important part of the inflammatory process in histoplasmosis and are regularly involved and caseated during the primary infection The lymph nodes can be very large in primary histoplasmosis and can liquefy and perforate into the bronchus just as tuberculous lymph nodes sometimes do The lymph node component parallels the development of the primary focus and generally one is impressed by the similarity of development of caseation or calcification which can be seen in the primary focus as compared with the satellite lymph nodes

Darling (6) mentioned fibrocaseous tubercles in bronchial lymph nodes but if one considers that his case may have had tuberculosis in addition to histoplasmosis one cannot accept the report

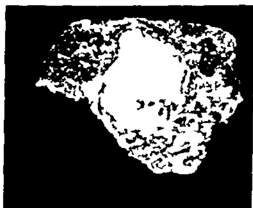


Fig 4 The growth of the primary lesion can be studied on the picture showing the incorporation of a small nodule which had formed outside the thin fibrous capsule of the focus into the main mass of necrosis and caseation (A 5513–2 3x) (Deeper cut of lesion seen in Fig 3)

as conclusive demonstration of a pulmonary primary histoplasmic complex It is also well to remember that Ghon s monograph (9) which made the concept of a primary complex known and scientifically acceptable appeared several years after Darling s first observation of histoplasmosis

Christie (3) observed in a ten months old infant two calcified nodules of 3-4 mm in diameter in each lower lobe with granulomatous lesions in the hilar lymph nodes Histoplasma was recovered by culture but no information was given as to whether it came from one or both lung lesions or from the lymph nodes Some doubt arises whether this really represents a primary focus since there were bilateral lesions Kuzma (10) described a caseous nodule in the lung but failed to mention the presence of satellite lymph nodes

Puckett (13) described two primary complexes but since the material was surgically removed it would be hard to prove that these are true primary lesions However the fact that peripheral caseated lesions with hilar lymph nodes existed strongly suggests that it really represented a primary complex

All of Puckett s (13) solitary lesions were subpleurally located and he expresses the thought that they represent primary lesions which have enlarged The distribution of his lesions is rather inter

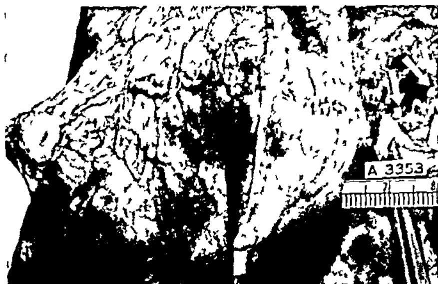


Fig 5 3353 Huge calcified subpleural primary focus with (calcified) pleural lymphangitis traceable to satellite lymph node containing calcific lesions (*arrow*)

esting right upper lobe—12 right middle lobe—14 right lower lobe—11 left upper lobe—12 left lower lobe—17 which very greatly simulates the distribution we have found in primary foci (see Table II)

Presently it is not known whether progression of the primary focus into chronic pulmonary histoplasmosis exists (but to carry on with the analogy to tuberculosis one has to assume so) In view of the extensive caseation in the center of some primary foci it appears likely that breakdown and cavitation occurs which then could be called a primary cavity analogous to similar happenings in tuberculosis On the other hand it is conceivable that some primary lesions develop into a so-called histoplasmoma We have seen pulmonary lesions which were rather large and had typical concentric lamination characteristically seen in histoplasmomas but which undoubtedly were primary lesions in view of the presence of satellite lymph nodes



Fig 6 314a Huge primary (calcified) focus of histoplasmosis with concentric layering reminiscent of structure seen in so-called histoplasmomas Massive calcification of regional lymph node

THE RECENT PRIMARY FOCUS

To judge from the relatively small accumulated experience the primary active lesion in histoplasmosis begins as a bronchopneumonia with phagocytic cells (containing the organism Histoplasma capsulatum) filling the alveoli In later stages the histoplasmic inflammation can exhibit a great variety of tissue response one can see only histiocytic proliferation or epithelioid cell tubercles with or without necrotic centers filled with polymorphonuclear leukocytes or areas of inflammation with leukocytes dominating the picture Mutatis mutandis the same is true in the specific case of the primary active lesion in the lung We have seen foci which on gross inspection appeared to be rather well circumscribed but not encapsulated and which had the yellow color of caseation Under the microscope such lesions blended slowly with the surrounding lung tissue without exhibiting the sharp limit suspected on gross appearance In early cases the lung had a peculiar type of desquamative inflammation with densely packed phagocytes in the alveoli

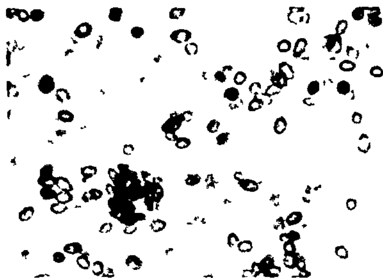


Fig 7 The primary calcified lung focus The Crocott silver stain reveal innumerable yeast cells in clusters (representing groups lying in phagocytes) and independently Notice fairly uniform size and oval shape (Crocott—1300×)

Histoplasma was present in the histiocytic elements The lung structure in some of these lesions showed little alteration and not only were the elastic fibers visible but many alveolar walls were clearly recognizable even on HE stain In more advanced foci necrosis ensues which however may be incomplete for considerable time Finally however the center of the larger lesions especially becomes completely caseated Polymorphonuclear leukocytes have in our experience never been a dominant feature which makes it different from the early primary lesion of tuberculosis The densely filled alveoli containing desquamated alveolar cells and histiocytes are indeed characteristic for histoplasmosis The formation of a capsule is not an early reaction and the slow fading out of the center of the inflammation is microscopically obvious even after many weeks of duration of the primary focus Lymphangitis becomes obvious microscopically and sometimes even grossly if the pleural lymphspaces are involved The satellite lymph nodes seldom show complete caseation and frequently microscopically show discrete

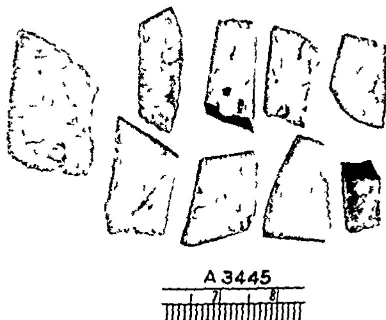


Fig 8 Numerous calcific foci in the spleen with Histoplasma capsulatum in the center are results and remnants of hematic spread during primary infection period Notice concentric structure of splenic lesions

epithelioid cell tubercles with or without central necrosis Seldom the number of recognizable fungi is great in the lymphadenitis If diffuse involvement of the lymph nodes occurs they become markedly enlarged and rather brownish yellow with an edematous appearance very different from the clear yellow of the tuberculous caseation

Whether there is progression of healing is regulated by immunologic processes which escape morphologic observation

The result of healing (the arrested primary complex) will be described in the following chapter

MORPHOLOGY OF THE ARRESTED PRIMARY COMPLEX

Already grossly there is some difference between the healed primary complex of histoplasmosis and tuberculosis In the latter the lesions are usually small and rather hard to find in many instances In tuberculosis it is not unusual to find calcific foci of

1 or 2 mm which can be located only after thorough palpation and/or multiple sectioning of pulmonary tissue occasionally only after x ray examination of the lungs which have been removed from the body In histoplasmosis the situation is quite different The foci in the lung and lymph nodes are very prominent measure frequently 0 5 cm up to 3 cm and more There is hardly ever a problem in locating the pulmonary or lymph adenitic component of the primary complex (2 17 18)

Another gross difference especially in the lymph nodes is in the composition of the lesion since in histoplasmosis numerous discrete chalky lesions are found (rather than diffusely calcified foci) On x ray this gives a stippled appearance which we consider rather characteristic for histoplasmosis (2 17)

Stains for connective tissue and elastic fibers reveal that complete destruction of pulmonary tissue is not invariable However to a great degree this is dependent on the size of the focus In the small lesions frequently complete destruction occurs whereas in the larger ones there is only partial and central destruction of the structure In the healed primary focus the center—which on microscopic examination contains the greatest number of organisms—is generally rock hard and needs considerable more time for decalcification than the comparatively less mineralized periphery

TABLE I

COMPARISON OF SIZE OF COMPONENTS OF PRIMARY COMPLEXES IN HISTOPLASMOSIS AND TUBERCULOSIS

	Total Number	*1-4 mm*	*5 10 mm*	*10 mm or more*
Primary Focus Lung				
Histoplasmosis	67	31%	66%	3%
Tuberculosis	34	9%	[illegible]0%	
Lymph Nodes of the Primary Complex				
Histoplasmosis	0	68%		3%
Tuberculosis	4	88%		1%

(Modified from Straub M and Schwarz J (18))

We have repeatedly observed active foci which had inflammatory changes in the periphery including tubercle formation and it appears obvious that this is the mechanism of growth of the lesions. Perifocal edema becomes inflammatory exudate, epithelioid cells develop, nodules form and the tubercle becomes caseated and later incorporated into the main mass of the primary focus. At this time apparently invasion of the lymphatics and blood stream occurs with great regularity. We have shown that calcific lesions in the spleen and to lesser degree in the liver can be found which can be explained only as hematogenous metastases (16). In several cases which we had the good fortune to examine the status of granulomatous response and caseation in the spleen was analogous to that found in the primary pulmonary focus and its satellite lymph nodes. Such active lesions in the spleen per se are microscopically indistinguishable from the caseated pulmonary or lymph node lesion.

The distribution of primary foci in the different lobes is quite congruent with similar findings in tuberculosis and runs closely parallel with the volume of each lobe. The available figures are too small to make a definite conclusion about the distribution within the lobes but we have the impression that just as in tuberculosis the lower two thirds of all lobes are more frequently the site of primary foci in histoplasmosis.

TABLE II

X-RAY MEASUREMENT OF INTRATHORACIC CALCIFIC FOCI

	5 mm or less	*6 mm or more*	*Stippled*
Positive histoplasmin negative tuberculin	1	30	75%
Histoplasma capsulatum demonstrated in focus	-	22	67%

(Modified from Serviansky and Schwarz (17).)

HISTOLOGY OF THE HEALED PRIMARY COMPLEX OF HISTOPLASMOSIS

Basically the lesions have to be divided into small and large foci since there is considerable microscopical difference between the two. The small focus of histoplasmosis demonstrates a structure very similar to the one seen in the primary focus of tuberculosis. The central area presents complete destruction but frequently instead of the dense calcification which is found in tuberculosis less heavy mineralization is seen in histoplasmosis. The normal structures are seldom completely destroyed including the elastic fibers. Organisms are found in the center sometimes in very large numbers. This small focus can become arrested and is surrounded by a narrow capsule of connective tissue which often includes a thin rim of bone generally with some bone marrow in it.

The large focus of histoplasmosis is more complex in its structure. *In the center one sees the lesions described in the small focus* that is more or less complete destruction of tissue including elastic fibers. However going towards the periphery there is a marked increase in connective tissue which is greater than one could expect in the average lung. Apparently this represents the end stage of a pneumonic process in which the exudate has become necrotic and later takes on calcium. This area of scarring which became calcified merges with a broad capsule surrounding the large focus which is fibrotic and frequently hyalinized. Vessels and other structures can be clearly recognized as shadowy remnants in this area. Calcification and ossification of this broad capsule is not unusual and the

TABLE III

Distribution of Primary Foci of Histoplasmosis in the Different Lobes of the Lungs

RUL	*RML*	*RLL*	*LUL*	*LLL*
11	7	15	14	21

(Modified from Straub and Schwarz (18))



Fig 9 485 The bony rim of the burned out primary lesion shows frequently bone marrow production The periphery of the focus is fibrotic or hyalinized and does not contain organisms (Van Gieson—160×)

bone which has been formed frequently has a double rim with mar row in between Occasionally the large focus shows complete de struction with a peculiar amorphous chalky material filling it up

The development of the large focus apparently occurs by incor poration of collateral inflammation This results in desquamative pneumonia with production of occasional tubercles which then caseate and are themselves incorporated into the main focus This repetitious process would explain the concentric rings seen in some of the primary lesions Each ring indicates the outer border of one period of inflammation with capsule formation The secondary incorporation of perifocal inflammation into the main focus then can form many such laminated rings Lymphatic spread from the focus obviously occurs and small satellite nodules are found in the tissue surrounding the main lesion The pleura over the focus is often thickened and completely adherent to the focus but extensive adhesions seldom are observed Large exudative (pleural) accumu lations seem to be unusual in primary histoplasmosis also The lymph node component of the primary complex is larger in histo plasmosis than in tuberculosis and the calcification has a less homo geneous and more discrete character The small portions of cal



Fig 10 2616 The arrested primary focus is large encapsulated with bone formation in the wall Incomplete destruction of elastic tissue alveolar walls and vessels demonstrated by special stain At this time all activity has ceased and the organisms found in the center of the focus cannot be grown on culture (Van Gieson Elastica—90×)

cium deposited are often surrounded by broad fibrous or hyaline bands There is much less anthracosis and anthracosilicosis in the lymph nodes of histoplasmosis than there is in tuberculosis A possible explanation is that the primary infection occurs in early age groups and the involved lymph nodes become scarred in histoplasmosis They consequently are eliminated from drainage and do not become anthracotic whereas at present tuberculous infection tends to occur in an older age group where many of the lymph nodes already have become anthracotic

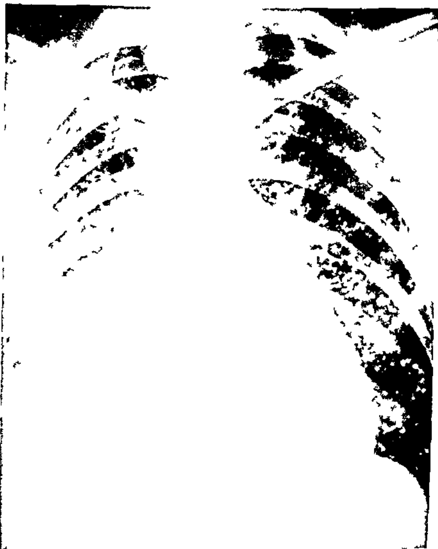


Fig 11 Multiple calcified lesions attributed to mitral stenosis Death three years after chest x ray was taken reveals hundreds of calcified round lesions some with clearly recognizable concentric lamination Histoplasma capsulatum demonstrated in almost every nodule which was examined This picture may well represent multiple primary lesions in a case of massive exposure (Courtesy of Dr Ben Felson)

Lymph nodes which often become very much enlarged during primary histoplasmosis naturally produce complications by perforation in the acute and during the chronic stage obstruction—partial or complete—of bronchi is seen by compression from without or by formation of broncholiths after partial irruption of calcified fragments of lymph nodes into the bronchus We have observed a lethal hemorrhage from the point of ulceration through the bronchial wall Bronchiectasis emphysema and atelectasis may result from the compression

An interesting feature is the presence of so-called histoplasmomas which as such were recognized first by Puckett (18) This author has expressed the opinion that these lesions possibly represent enlarged primary foci In favor of such an opinion are several facts First the distribution is quite similar to the one found by us in the healed primary foci Secondly several of our primary lesions are large enough to fall into the category of histoplasmomas and show the same laminated structure which is so characteristic of the histoplasmoma In addition in many cases where histoplasmomas are discovered radiologically hilar lymph nodes can be found at surgery or on x ray Microscopic examination of primary foci reveals pathology in the same general developmental stage as is found in the so-called histoplasmoma However this is probably not the only or even most frequent pathogenesis of histoplasmomas it is just one of several possibilities

In tuberculosis—where a considerably larger experience exists—the occasional occurrence of multiple primary foci (19) has been well documented and there can be little doubt that similar happenings will be observed in histoplasmosis In fact the findings observed in epidemics well may represent such instances of extremely multiple primary foci Examination or such a case revealed multiple lesions of 3 mm diameter of more all calcified with the structure consistent with healed primary foci The rather unique occurrence of massive inhalation of spore containing dust in such individuals could well be consistent with the anatomical finding of numerous (dozens or hundreds) pulmonary lesions in the same state of development Naturally lesions of equal size could be hematogenous in origin however the epidemiologic evidence

and clinical picture indicate rather that the fairly large foci are results of inhalation—infection because they become visible a short time after exposure (a few days to a few weeks) They are soft at first and calcify however this may take many years (7) The presence of calcification indicates previous necrosis which is more likely to occur in primary histoplasmosis This individual healed lesion of epidemic histoplasmosis is indistinguishable from the classical solitary healed primary focus as described above

RESUMEN

It has been firmly established that the usual route of entrance of Histoplasma capsulatum into the body of humans and lower animals is through the respiratory tract The facts pointing in this direction are the following (1) epidemiologic evidence especially focal outbreaks (epidemics) which are extensively discussed in the corresponding chapter (2) the presence of active primary complexes in acute cases of histoplasmosis at autopsy (3) x ray evidence of healed calcific pulmonary lesions the histoplasmic nature of these was verified by Straub (18) who demonstrated the presence of primary complexes with visual demonstration of Histoplasma in the arrested lesions in 70 cases out of a total of 105 unselected autopsies in Cincinnati

Theoretically other portals of entrance are possible there is no reason why occasional enteric infections or inoculation into the skin or into mucosae communicating with the external environment could not occur If such infections at all happen they must be exceptional and should be accepted as primary only if a demonstrable primary chancre with satellite lymph nodes develops (primary complex) This in the case of enteric primary infection would be hard to demonstrate clinically but considerable experience with autopsies in the endemic area failed to reveal any appreciable number of mesenteric calcifications in the absence of thoracic calcifications A possible primary chancre on the penis has been described (5) The mucosal lesions observed in the naso pharynx and mouth of elderly persons are unquestionable secondary to pulmonary infection which has been demonstrated time and again

(1) Only simultaneous trauma and introduction of Histoplasma could conceivably produce a primary oral lesion

The primary active lesion becomes necrotic caseated and arrested in the overwhelming majority of cases The primary focus of histoplasmosis is often large and becomes irregularly calcified (stippled) The primary infection produces with regularity a primary complex consisting of the primary (pulmonary parenchymatous) focus with large satellite lymph nodes which are generally very prominent and may overshadow the volume of the primary pulmonary lesion

REFERENCES

1 Baum G L, Schwarz J Bru n Slot W J and Straul M Mucocutaneo h topla mosis *A M A A ch Dermat 76* 41 19

2 Bronson M and Schwarz J Roentgenographi pattern n hi topla mo *Rev Tuberc* 6 1 3 131 19 7

3 Christie A Hist plas n sis an l pulmonary c l ificat ons *An New Yo k A ad Sc 50* 1 83 1 98 19 0

4 Christie A and Peterson J C Pulm nary calc ficati n in negat ve re ctor t tuberculin *Am J Pub Health 35* 1131 114 1945

5 Curtis A C and Cawley F 1 Genital histoplasmosis *J U ol 57* 81 8 194

6 Da ling S T A proto n general infect on producing pseudotube cles in the lungs and focal necroses in the liver spleen and lymph nodes *J A M A 46* 1283 1 8 1906

Felson B Jones G F and Ulr h R 1 Roentgenolog c a pects of diffuse milı ary granulomat s p eumon t of unknown etiology *Am J Ro ntgen l 64* 740 746 19 0

8 Fu colow M L Development of calcification n pulmonary les ons assoc ated with sen it ity to h topl smos *Pub Health Rep 64* 1363 1393 1949

9 Ghon A *D r p ima e Lu g nle d be der Tube kulose de K nder* W en Urban 191

10 Kuzma J F H stoplasmos pathologic and cl n al find ngs *D Chest 13* 338 344 191

11 Palmer C E No t be c lous pulmon ry calcificat on and sensiti ty to hi to pl smin *Pub H lth Rep 60* 513 5 0 194

12 Parrot M J *C mpt nd So B ol* 8 308 18

13 Pu kett T F P lmon ry hi toplasmos a study of 2 cases w th identification of H cap ulat im n es t d les ons *Am Rev Tuberc 67* 4 3 4 6 1953

14 Schulz D M A p t lly h led prima y les on n a case of general d h to pl m s s *A ch P th 50* 4 463 19 0

and clinical picture indicate rather that the fairly large foci are results of inhalation–infection because they become visible a short time after exposure (a few days to a few weeks) They are soft at first and calcify however this may take many years (7) The presence of calcification indicates previous necrosis which is more likely to occur in primary histoplasmosis This individual healed lesion of epidemic histoplasmosis is indistinguishable from the classical solitary healed primary focus as described above

RESUMEN

It has been *firmly established that the usual route* of entrance of Histoplasma capsulatum into the body of humans and lower animals is through the respiratory tract The facts pointing in this direction are the following (1) epidemiologic evidence especially focal outbreaks (epidemics) which are extensively discussed in the corresponding chapter (2) the presence of active primary complexes in acute cases of histoplasmosis at autopsy (3) x ray evidence of healed calcific pulmonary lesions the histoplasmic nature of these was verified by Straub (18) who demonstrated the presence of primary complexes with visual demonstration of Histoplasma in the arrested lesions in 70 cases out of a total of 105 unselected autopsies in Cincinnati

Theoretically other portals of entrance are possible there is no reason why occasional enteric infections or inoculation into the skin or into mucosae communicating with the external environment could not occur If such infections at all happen they must be *exceptional and should be accepted as primary only if* a demonstrable primary chancre with satellite lymph nodes develops (primary complex) This in the case of enteric primary infection would be hard to demonstrate clinically but considerable experience with autopsies in the endemic area failed to reveal any appreciable number of mesenteric calcifications in the absence of thoracic calcifications A possible primary chancre on the penis has been described (5) The mucosal lesions observed in the naso pharynx and mouth of elderly persons are unquestionable secondary to pulmonary infection which has been demonstrated time and again

THE PATHOLOGY OF CIRCUMSCRIBED LOCALIZED LESIONS OF HISTOPLASMOSIS

THOMAS F. PUCKETT

It is possible that as many as one fifth of our population is or has been infected with Histoplasma capsulatum and that the number of new cases annually may approach one half million.

The vast majority of these patients have an uneventful, uncomplicated and often relatively, if not completely, asymptomatic course and the disease escapes detection during its active phase. A moderate number of those infected are left with residual intrathoracic lesions in the form of one to many discreet, scattered fibrotic or, more often, calcified areas in one or more lobes that are seen in roentgenograms taken in surveys or for other purposes. One common type of lesion that is frequently a residual of histoplasmosis is seen by x-ray as a solitary intrapulmonary "nodule" and has been referred to by clinician and roentgenologist under a host of different designations. The term "histoplasmoma" has been used to designate this type of residual lesion (1) because of its similarity to some lesions due to M. tuberculosis and C. immitis for which the term "tuberculoma" and "coccidioidoma" have come into common usage.

The solitary localized lesion shows no significant predilection for any given lobe (2, 3). In the present writer's series of 66 residual parenchymal lesions, more than one half of which were of the solitary type, twelve were in the right upper lobe, fourteen in the right middle lobe, eleven in the right lower lobe, twelve in the left upper lobe and seventeen in the left lower lobe. Unquestionably some of these lesions are the parenchymal components of a primary complex.

Schulz (4) has described the primary complex of histoplasmosis in infants and Straub and Schwarz (5) have given a detailed

15 Schulz D M Histoplasmosis a statistical morphologic study *Am J Clin Path* *24* 11 26 1954

16 Schwarz J Silverman F N Adriano S M Straub M and Levine S The relation of splenic calcification to histoplasmosis *New England J Med* *252* 887 891 1955

17 Serviansky B and Schwarz J Calcified intrathoracic lesions caused by histoplasmosis and tuberculosis *Am J Roentgenol* *77* 1034 1041 1957

18 Straub M and Schwarz J The healed primary complex in histoplasmosis *Am J Clin Path* *25* 727 741 1955

19 Terplan K Anatomical studies on human tuberculosis *Am Rev Tuberc* (suppl) *42* 1 176 1940



Fig 1 Histoplasmoma Note pleural plaque keystone laminated appearance and central necrotic focus

It is most important that this characteristic central area be searched for and sectioned for it is here that organisms will be found in the greatest numbers and often they can be found in no other areas (1 3)

Microscopic examination shows little variation from lesion to lesion Typically the lesion is surrounded by normal lung parenchyma which may or may not show increased thickness of the interlobular collagenous fibers There may be an occasional epithelioid tubercle in close association with a small vessel but in general the surrounding parenchyma is normal The lesion is surrounded by a zone of lymphocytes of varying thickness that may be missing

account of the primary complex as seen in their autopsy material There is evidence however that reinfection type disease may occur (5) It is possible therefore that the circumscribed lesions described may in some instances represent reinfection

GROSS

The circumscribed localized lesion of histoplasmosis varies from 0 5 to 3 5 cm in diameter is usually round but may be ovoid Its consistency varies from stony hard to firm and rubbery Typically it is located in the immediate sub pleural region and is associated with a dense round oval or stellate pleural plaque that is fused to one edge of the lesion The color of the plaque varies from pinkish gray to yellowish tan and it is sharply demarcated from the adjacent normal pleura There may be a number of 0 1 to 0 2 cm grayish nodules within the adjacent pleura that represent lymphatic spread

The cut surface shows the typical lesion to be sharply demarcated from the adjacent normal lung with a smooth even border (Fig 1) Occasionally there are 0 1 to 0 2 cm pearly gray lesions adjacent and occasionally there are grayish bands representing thickened interlobular septa In some cases there may be thickened bronchioles and prominent thick walled small vessels but usually the adjacent lung is normal

The cut surface usually shows the pleural plaque to extend into the main lesion as a wedge simulating a keystone Anthracotic markings at the edges of this triangular mass are often prominent in the older lesions Older lesions tend to show prominent grayish white concentric markings creating a laminated appearance with prominent anthracotic deposits between the laminations Younger lesions have a more homogenous appearance and a more grayish tan to grayish pink color Without exception multiple thin slices will show a small focus usually central that presents a granular appearance in contrast to the adjacent portions Usually this focus is softer sometimes contains frankly granular necrotic material but occasionally is soft and rubbery It is never liquid nor does the cut surface of the lesion present the ragged moth eaten appearance often seen in similar lesions due to Coccidioides immitis

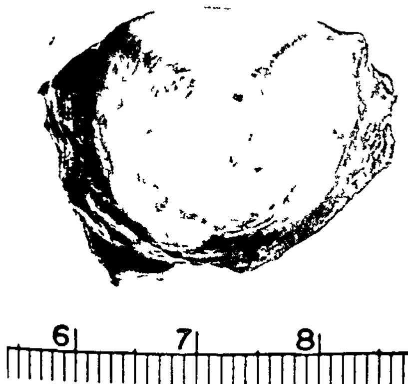


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over a large segment and usually shows a tendency to form accumulations rarely with germinal centers The peripheral portion of the lesion consists of dense collagenous tissue with scattered plump fibrocytes Blood vessels and bronchioles are often in intimate association with this layer but are rarely incorporated within it are usually pushed aside or distorted by its presence Central to this capsule there is a thin zone of loosely arranged large epithelioid cells although this zone is sometimes very scant or may even be absent in large segments Both Langhan s and foreign body type giant cells are usually present in this portion but are ordinarily sparsely scattered and they may be entirely absent especially in older lesions The peripheral eosinophilic infiltration so common in and adjacent to coccidioidomas is not present in similar lesions due to H capsulatum

Central to the zone of epithelioid cells there are broad acellular collagenous fibers making a capsule of varying thickness which enclose the soft granular or rubbery central portion that is readily seen on the cut section In the peripheral portion of this granular area may be seen concretions of various types including flecks of calcium indeed the entire area may be heavily calcified Straub and Schwarz (3) describe transformation to bone with marrow spaces in their autopsy cases Some cases show retention of the alveolar framework with the proper counterstain or with elastic tissue stains Calcification is always less prominent in these cases

The only decisive microscopic characteristic of the localized lesion of histoplasmosis is the presence of H capsulatum and it is in the central necrotic area that by the use of appropriate stains the organisms can be found

The present writer prefers the periodic acid Schiff stain (PAS) (6) simply because his original work was done with this stain it is easily prepared easily utilized affords good contrast and does not distort the morphology Two other stains have been used with equal enthusiasm by others and quite possibly offer advantages over the PAS stain (7 8) One cannot over emphasize the need for special staining procedures however Histoplasma may readily be seen in ulcerative mucosal lesions in the Kupfer cells in animals experimentally inoculated in histiocytes and elsewhere with

routine H & E stains. It has been the experience of the present writer and others that they cannot be seen in the granulomatous lesions stained by routine H & E stain (1, 9). They can be made out fairly well in stains for acid-fast bacilli that use a methylene blue counterstain but identification is difficult and the procedure is not satisfactory for diagnostic purposes.

With the use of the PAS stain one can often find myriads of organisms singly and in clusters in the soft centers of the localized lesion (Fig. 2). Usually there are great numbers of organisms that have the typical morphology, show little variation in size and shape, and they may be present in clusters. Frequently they are in association with many atypical forms that are probably degenerating forms. The identification and diagnosis is based upon identification of the characteristic organisms; however, as it has been the experience of the present writer and others (1, 3) that they cannot be cultured from the circumscribed localized lesions, it is therefore assumed that they are non-viable, certainly by present cultural methods.

There are frequent artifacts that are present in and near the soft central focus in the circumscribed lesion. Their number and type probably depend upon the age of the lesion. These are concretions of various types, some having a central "nucleus" and stellate "cytoplasm" that takes the red stain of the PAS and looks very much like an organism but not like H. capsulatum. Others have a darkly staining outer portion that takes a heavy counterstain and resembles a yeast-like cell.

The morphology of the organism is now well established and careful study will disclose that the artifacts do not present a serious handicap. With properly stained material, patience, and the use of the high dry or oil immersion lens, sufficient numbers of typical organisms can be found so that the diagnosis need never be made on the basis of a few suspicious structures.

The incidence of this type of residual lesion is not known. The incidence of histoplasmosis will vary according to the geographical area from which patients are selected. Straub and Schwarz (3) studied the lungs from 105 consecutive autopsies on patients dying from diseases other than histoplasmosis and were able to demon-



Fig 2 H capsulatum in necrotic focus (PAS stain x1650 slightly enlarged)

strate histoplasmic lesions in 50% of the 30 Negroes and 73% of the 75 white patients in the series These writers were primarily interested in a morphological study of the primary complex of histoplasmosis but it is noted that in 41 cases the parenchymal component was larger than 0 5 cm therefore might be classed in the category under consideration here

In the present writer s series of 66 cases of resected residual lesions more than one half were circumscribed localized lesions (2)

In Davis Peabody and Katz s series of 215 solitary pulmonary nodules (9) selected by very rigid criteria 82 or 38% were found to be granulomas and they were able to identify H capsulatum in 39 cases or 55% of these On the basis of their study one can say that about 18% of all solitary pulmonary nodules less than 6 0 cm in diameter are residual foci of histoplasmosis

Moreover it has been shown by Zimmerman (10) that H capsulatum could be demonstrated in many lesions that had previously been designated as tuberculomas on the basis of their morphological and histological appearance rather than the presence of acid fast bacilli in the lesions It is therefore evident that the solitary localized lesion of histoplasmosis is a very common lesion indeed

REFERENCES

1 Puckett T F Pulmonary histoplasmosis study of twenty two cases with identification of H capsulatum in resected lesions *Am Rev Tuberc 6* 4 3 19 3

Puckett T F Pulmonary histoplasmosis *A J Surg 20* 9 19

3 Str l M and Schwarz J The healed primary complex in histoplasmosis *Am J Clin Path* 5 19

4 Schulz D M Histoplasmosis a statistical review *Am J Clin Path 24* 11 19 4

5 Zeidberg L D Dillon A and Ga R S Some factors in the epidemiology of histoplasmin sensitivity in Williamson County Tennessee *Am J Pub Health 80* 41 19 1

6 Kligman A M and Mescon H The periodic acid Schiff stain for the demonstration of fungi in animal tissue *J Bact 60* 41 1950

7 Gridley M F A stain for fungi in tissue sections *Am J Clin Path 23* 203 19 3

8 Grocott R G A stain for fungi and tissue sections using Gomori s methenamine silver nitrate technique *Am J Clin Path* 5 9 1955

9 Davis E W Peabody J W and Katz S The solitary pulmonary nodule *J Thoracic Su* 3 8 1956

10 Zimmerman L E Demonstration of histoplasma and coccidioides in so called tuberculomas of lung Preliminary report on thirty five cases *Arch Int Med 94* 690 1954

THE GENERAL PATHOLOGY OF HISTOPLASMOSIS

Henry C. Sweany

It required over forty years for many of the clinical aspects of histoplasmosis to be recognized and because the disease was usually benign with a scarcity of pathological material there was until recently a meagre knowledge of its pathology. Only within the last few years have the more important phases of the pathology been observed and recorded. As the knowledge of the disease unfolds new aspects appear that require special consideration. One of these is the primary complex which resembles the primary infection of tuberculosis. Another is represented by the so called coin lesions. Both of these types are handled in separate chapters. When more is known of the disease it may be found necessary to include these groups with general pathology but for the present it is more convenient to class them separately.

For this chapter we shall consider what is known of the general pathologic features of the disease as it disseminates, localizes or heals in the human body.

Darling's first descriptions (1) were those of a dissemination involving almost exclusively the reticulo endothelial cells of the body. There were localizations in the other parenchymatous organs but his description of the fulminating type with the morphology of the parasites may still be considered as classical. Others to follow added little until the more chronic forms of the disease were recognized.

One of the recent studies to emphasize the importance of pathology and attempt to classify the disease according to pathology was that of Schwarz (2) who described three types of disseminated histoplasmosis. One where the parasites were found in histiocytes but where there was no collateral tissue reaction. In a second group there was a marked proliferation of histiocytes in the spleen, lymph

nodes and bone marrow but still there was little or no necrosis This corresponds to the acute disseminated phase In a third group there was a granulomatosis where there was evidence of a chronic granuloma with or without caseation In fact this group was divided into exudative and proliferative as Aschoff did long ago for tuberculosis In this last group the lesions may be found in other organs such as the lungs the liver and adrenals Nodules of epithelioid cells with the Langhans giant cells were frequently present In the centers of the nodules caseation existed and contrary to the observations of later workers he claims that polymorphs were common especially in the lungs where necrosis and abscess formation dominates the picture The caseation is most noticeable in the adrenals Then there are mixtures of several of these types that may be found

Puckett (3) paid special attention to the large round lesions which had been called tuberculomata but he must be given credit for finding that out of 22 cases all of them contained H capsulatum as shown with the PAS stain Of these 22 cases 7 represented a focal encapsulated pneumonitis 9 were made up of large encapsulated lesions with several daughter colonies or satellites four had large encapsulated lesions along the lymph nodes only one was cavitary and one involved only the hilum lymph nodes Zimmerman (4) made similar studies and found 18 of 35 of these round lesions which had been called tuberculomata to be histoplasmosis The relationship of these special forms of histoplasmosis to primary infection or re infection is still unsettled (See also Figs 6 11 Chapter 13)

One of the most extensive studies in the pathology of histoplasmosis has appeared within the last two or three years under the authorship of Chapman H Binford (5) of the Armed Forces Institute of Pathology in Washington D C While these cases were not all of the fulminating type most of them were generalized and the general pathologic findings have been well documented

Various tissue reactions have been particularly well described and the result has gone a long ways to clear up the cytological changes that exist in the disease Binford has taken up the tissues involved and has to a large extent given the incidence of the various

Fig 1 (A) Solitary fibrocaseous nodule in lung H & E x10 AFIP Acc 127350 (B) Numerous Histoplasma organisms in necrotic center of nodule shown in (A) Gridley fungus stain x1250 (C) Spleen AFIP Acc 119989



tissue changes in the series of cases that he has described He describes the histiocytomycotic lesions in which he points out that H capsulatum has a predilection for the reticulo endothelial system He states that the yeast form grows prolifically in the cytoplasm of the histiocyte and greatly enlarges the organ involved In certain lesions there is a caseous necrosis around which are palisaded spindle shaped cells that form the margin of the necrotic areas In 19 of these 22 cases this histiomycotic response was observed In 11 the reaction occurred alone and in 8 it was mixed with granulomatous lesions

A most important observation and one that has been verified by others is that neutrophils plasma cells and lymphocytes play little or no role in histoplasmosis Epithelioid cell granulomas were common and in the solitary or multiple pulmonary nodules and in 10 of 22 disseminated cases these epithelioid granulomas with Langhan s giant cells were present Since many of these centers exhibited caseous necrosis it was difficult to distinguish the nodules from tuberculosis On the other hand the non-caseous epithelioid cell granulomas mainly sarcoidosis had made it difficult to separate from that disease

In these non-caseous granulomas the fungus cells were seldom seen on the H & F stain but the Gridley stain permitted the author to find a few cells of H capsulatum in the sarcoid type of lesion In one case a stained specimen of the axillary lymph nodes revealed H capsulatum when the diagnosis had been sarcoidosis

Another peculiar observation was what was called organizing pneumonitis where there had been a filling of the alveoli with fibrin and a partially organized fibrous plug was present with an involvement of alveolar walls in the process H capsulatum was

Upper A few Histoplasma organisms in a granuloma Gridley fungus-stain x1950 The spleen weighed 575 Gm and the original diagnosis was miliary tuberculosis or sarcoidosis *Lower* Epithelioid cell granuloma histologically compatible with sarcoidosis H & E x(3 (D) Adrenal medulla demonstrating failure of Histoplasma in necrotic tissue to stain with hematoxylin and eosin AFIP Acc 579628 *Upper* Periodic acid Schiff method light green counter stain x1250 *Lower* Hematoxylin and eosin stain x1250 (Used by permission of the *Am J Clin Path* the author Chapman H Binford and the Armed Forces Institute of Pathology Washington D C)

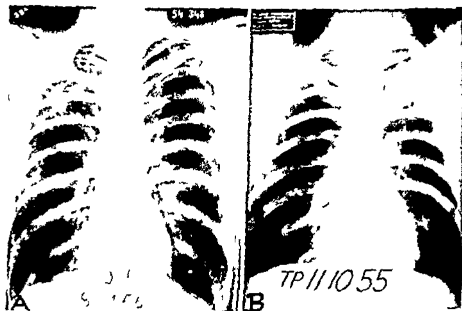


Fig 2 (A) Roentgenogram of D H L21 No 25 189 taken on August 11 1955 showing a fibroid and partly infiltrative lesion in right subapex (B) Roentgenogram of J J B23 No 25 2_3 taken on November 10 1955 showing a more inflammatory type of lesion in the left subapex (*Used by permission of Dr J A Myers Editor and Diseases of the Chest*)

readily demonstrated in such lesions (See Fig 1(D) Chapter 13)

In a subcutaneous lesion Dr Binford found a spindle cell type of reaction that resembled fibrosing granulomatous tissue The author also mentioned the round lesions that have been so well described by Puckett (See Fig 1(C) Chapter 13)

The author then takes up a few special organs and mentions them because of the unusual features observed in the disease process in these organs In 21 of the 22 cases the adrenals were involved The weight of the glands on some of these cases went up to 100 gm The author mentioned the fact that this is a well known observation and it seems to bear out what others have also found Not infrequently these cases end up in an Addisonian type of collapse Although the author does not mention the number it is assumed from the text that all the spleens were involved by packing the reticulo endothelial system with parasites resulting in a tremendous

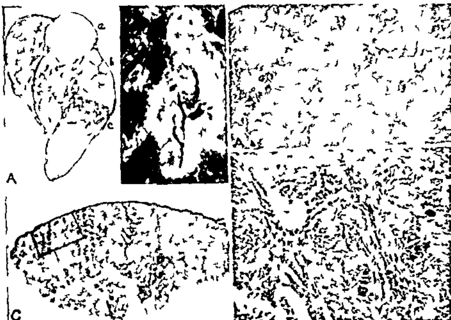


Fig. 3 (A) *Upper left* A sketch of re ected upper lobe of T I B23 No 9,993 Figure 9(I) purporting to show the cystic protrusion at a the chronic pneumonia at b and the atelectasis at c (B) *Upper center* A slightly enlarged caseous focus from the consolidated region below the cystic cavity with early cavity formation H & E x4.5 (C) *Lower left* A low power photo of section of the chronic pneumonitis t b in (A) H & E x4 5 (A) *Upper right* A higher magnification of area marked off in (C) H & E x90 (B) *Lower right* A still higher magnification of an area of the chronic pneumonitis Note the imperfect giant cells fibroblasts fibrosis macrophages and the few lymphocytes with no polymorphs H & E x140 (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

enlargement of the organ He did mention that 6 of the 22 had a granulomatous type of lesion resembling Boeck's sarcoid but in which H capsulatum were readily found Three cases had medullary and papillary necrosis of the kidney as did 3 cases with vegetative endocarditis In these last two groups there were bizarre shapes of the parasites and not only were they large in size but there were many mycelia and many cells in which attempts of mycelia

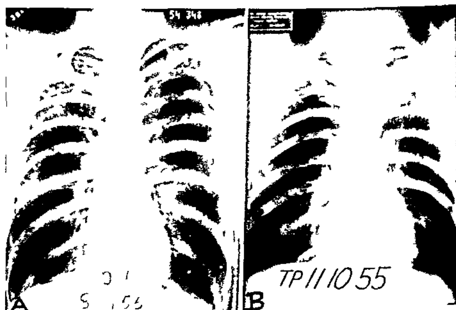


Fig 2 (A) Roentgenogram of D H B21 No 25 139 taken on August 11 1955 showing a fibroid and partly infiltrative lesion in right subapex (B) Roentgenogram of T P B23 No 25 923 taken on November 10 1955 showing a more inflammatory type of lesion in the left subapex (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

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or as it is modified by various factors of resistance of the host or virulence numbers and/or peculiarities of the parasite For example the round lesions are isolated types that still have not been connected up definitely to the other types of pathology The question arises whether they are part of an infiltrate or of an expanding process from a small focus whether they are primary or re infection lesions or both

Some of these problems will be discussed in attempt to make the knowledge of the pathology of the disease more complete Most of the material submitted has been studied at the Missouri State Sanatorium at Mt Vernon Missouri (7 8) Since the material at hand was almost exclusively confined to the chest and consisted in resected lesions there must needs be certain unavoidable omissions

The first tissue reaction to the parasite of histoplasmosis like that of most toxic substances including tuberculosis has been shown by Procknow to be neutrophilic This phase apparently soon passes from the tissue reaction for most of the disease process is confined to the reticulo endothelial apparatus so well shown by Schwarz (2) and Binford (3) Although Schwarz mentions neutrophilic reaction in certain lung lesions in general polys are rarely present except perhaps in mixed infections One of the main characteristics of the pathology of histoplasmosis is a general absence of polymorphonuclear leukocytes in the preponderant majority of lesions

Without attempting to trace the lesions throughout their course it will be sufficient to describe the types of gross pathology

First should be considered the earliest recognizable gross lesions namely the infiltrative focus (See Fig 2B) It is impossible to foretell the possible outcome of any focus that is found in routine gross section work Some may be the beginning of a primary lesion that will expand to different sizes extend to a lymph node and or go on and become generalized if not fulminating in character Some may be reinfection infiltrates Be that as it may the focus soon shows necrosis in the center and with a ring of epithelioid cells generally placed in palisading fashion around the circumference as Binford has described In the caseous center there is not the

formation were just beginning In 15 cases having brain sections only 2 abscesses of H capsulatum were found

Although the author was careful to mention the fact that the report was not complete for every organ it gave a very fair idea of what to expect in the line of cellular pathology in progressive histoplasmosis Perhaps one of the most obvious missing features was any mention of involvement of the liver It seems rather strange that of 22 cases having a disseminated disease some would not have involved the liver

The last of the article takes up the many variations observed in the parasite as it was found in various organs As mentioned before giant forms mycelial forms and various other interesting aberrations of the parasite were noted

Vivian and associates (6) reported on 20 cases from the Mayo Clinic After listing clinical symptoms of sore throat in 11 cases chest pain productive cough loss of weight fatigue fever chills sweats and anemia they recorded 7 of 9 cases of hepatomegaly and 4 of 7 cases of splenomegaly before necropsy The pathologic data was based mostly on necropsy findings or very suggestive clinical findings In 14 cases there was pulmonary involvement in one the mediastinal nodes were involved with Hodgkin s disease histoplasmosis or both the pharynx liver and spleen were each involved 7 times the adrenals were involved in 6 cases and in another there was clinical evidence of involvement the bone marrow was involved in 5 cases and in 3 it was the first tissue to yield positive evidence of disease

From the preceding reports we can see that there are many clear-cut types of cellular pathology typical of histoplasmosis with only sketchy accounts of the gross lesions especially in a great number of the organs of the body It is clear however that there are certain disconnected aspects of the gross pathology of the disease Even in the microscopic lesions there are several well described types in which the findings are definite and the descriptions are precise but the relationships to other types of pathology are probably not definitely established As stated in the chapter on pathogenesis there are also gaps in the pathogenicity of the disease or the disease in motion as it progresses from one stage to another



Fig 1 (A) A low power view (x6) of the fibroid cavities shown in Figure 2(A) H & E x6 (B) A higher magnification of wall of cavity marked off in (A) H & E x36 (A) A still higher magnification of wall of cavity of (A) Note palisade arrangement of cell H & E x160 (B) An area of chronic pneumonia in lung tissue showing alveoli packed with organizing fibrous exudate with alveolar cells and macrophages H & E x 13_ (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

of the lesion Every lesion of this nature must be looked upon as one stage of evolution of the infection either spreading or static with probable termination in an excavation

The parasites in such a lesion are usually present but the location and numbers are variable Some lesions may reveal typical yeast bodies approximately one to two per high power microscopic field In other lesions the yeast may be difficult to find Sometimes

finely mixed debris of a tubercle with pyknotic nuclei but it is more of a coarse necrosis of tissue cells This is then what might be considered the elementary gross unit of the lesion of histoplasmosis The size may vary from a few millimeters to several centimeters depending on such factors as number and virulence of the parasites and the resistance of the host Although it is possible that these foci may localize any place in the lungs the common form encountered is found in the upper parts of the lungs just as is tuberculosis The question of the primary nature of the process cannot be determined without a corresponding conversion of the histoplasmin skin reaction At the present time it is very difficult to make this observation Of course an involvement of the lymph nodes followed by generalization may be considered as highly suggestive of primary infection but there is no reason now known why a re infection cannot become generalized also by way of the lymphatics There is one condition that is frequently present more than in tuberculosis and that is that uninfected adults may come in from an area free of histoplasmosis and be infected for the first time This situation practically never holds for tuberculosis because there are very few places on the earth where a considerable amount of tuberculous infection does not exist and in some places it is almost universal For the present this problem with its bearing on the pathology must be held in abeyance

Another unknown factor is relationship of the first focus to the slow growing or stationary round lesions described by Puckett Perhaps the resistance or lack of it with the virulence of the parasite may enter into the course of process

It seems clear that the acute massive infiltrative lesion of 5 to 10 cm size should not be classed with the fibroplastic round lesions They are entirely distinct Most of them show as soft caseous masses Perhaps most of them shell out into cavities which have so many varied characteristics as the process progresses The type described by Puckett are mostly balls of fibrous tissue with varying sizes of caseous foci Usually the caseous foci are scarce

The large infiltrate is composed of a coarse granular necrotic type of caseation with or without a fibrotic capsule depending perhaps on the age of the process or the progressive or stationary nature

they are located in some densely caseous center In others they may be nests of two to four small parasites in a matrix with granules of an undetermined nature throughout Some granules may appear in the ghosts of old yeast bodies In general this is the acute infiltrative lesion as distinct from the chronic round lesion in which the parasites are sometimes difficult to find The relationship of these two types of lesions is not known at the present time

The number of calcific lesions which develop into healing and encapsulated foci is another unknown but in the chapter on the primary lesion by Schwarz these lesions are well shown and perhaps all primary foci go through the stages of encapsulation calcification and ossification as in tuberculosis The lesion that progresses through ulceration may show a stellate crevass that finally penetrates a bronchus following which the caseous debris excavates leaving first a cavity with an uneven or irregular wall There is no pyogenic membrane of polymorphs and monocytes as in tuberculosis but a barren wall of coarse caseous debris largely of destroyed tissue cells Slight calcification may be present The fibrotic wall may be of a varied thickness depending on the age and chronicity of the process and the thickness of the wall may be from a few millimeters to many centimeters depending on the size of the caseous focus and age of the process

The wall of the cavity is usually made up of a dense hyalinized fibrous tissue with a periphery of macrophages in which no parasites may be seen From the inner wall there is a sloughing debris of coarse caseous material that gradually is transformed into a layer of fibroblasts and fibrous tissue that gradually develops a slick surface smooth as a mucous membrane Finally epithelization gradually takes place on this membrane This is a characteristic that sets most cavitary lesions of histoplasmosis apart from those of tuberculosis that is tuberculosis where no drug treatment has been given The untreated tuberculosis almost always has a pyogenic membrane even in the late stages of healing In advanced disease of histoplasmosis there are recent infiltrates beneath the cavities

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Fig 578 A high power microscopic view of center of caseous mass showing large yeast bodies about 3 to 5 microns in diameter B M S x1066 (B and C) *(Lower left and right)* Another view showing birefringence in (C)



Fig. 5. (The lesion from which this specimen was taken is shown in the x-ray in Chapter 22, Figures 1 and 2, by Dr. John Folk.) (A) *(Upper)* B 30 F B →


a
b
c
d
A
B



Fig 6 (A) Roentgenogram of S R P10 23 637 taken on August 21 1953 Note destroyed right upper lobe (B) Sagittal section of upper lobe of lung removed on September 25 1953 showing extremely thick walled cavities with no pyogenic membrane (Used by *permission of Dr J A Myers* Editor and *Diseases of the Chest*)

with some cavities showing clearing others partially cleared and still others near the apex of the lung which are completely cystic in nature

Sometimes the disease may become rapidly progressive and the cavity enlarged One example in our work was in a man who was reported to be an alcoholic where the lesion that was followed for over ten years as a moderately advanced and healing type of tuberculous process suddenly began to spread and the process did not stop until most of the parenchyma of the lung had all

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Fig 7 (A) Sections a b c and d made from encapsulated lesions removed and stained in 1956 H & E x 45 (B) Microscopic photograph of yeast bodies found in the c lesion of (A) G M S stain x 1066 (Used by permission of *Dr J A Myers Editor* and *Diseases of the Chest*)



Fig 9 (A) A low power view of dense ossified parenchymal lesion shown in Figure 8(A) There was also a lymph node lesion left behind perhaps the lymph node component of an old primary Small dots encircle several areas in which yeast bodies are found H & E x4 5 (B) An enlarged area outlined in black ink Note the bone and marrow (C) Microscopic view of yeast bodies found in area outlined by dots in (A) G M S stain x1066 (D) Another field outlined by the dots in (A) G M S stain x1066 (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

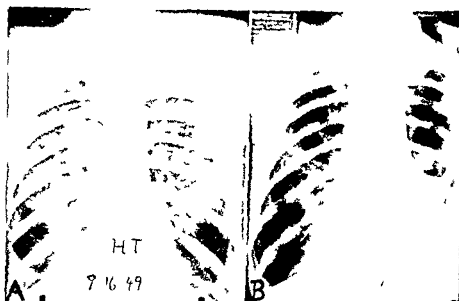


Fig 8 (A) Roentgenogram of H T B28 No 26 098 taken on September 16 1949 showing a fibroid apical lesion in the left apex and a few nodules below It was called a moderately advanced tuberculosis with an apical cavity (B) Same case taken on August 31 1956 showing what was thought to be a pneumothorax because of the fibrin body showing Instead it was found to be a completely destroyed lung (Used by permission of Dr J A Myers Editor and *Diseases of the Chest*)

excavated all the way to the diaphragm leaving a thickened pleura and a mass around the hilum made up of large bronchi and blood vessels About a dozen of these bronchi opened out into the cavity *A few parasites were found in the cavity wall and one culture of the* sputum was positive enough to make a diagnosis The old calcified foci which were present revealed many pockets of typically appearing yeast bodies within the center of the lesion These foci were not only calcified but they showed many rings of bone indicating that the process was quite old The relationship of these encapsulated lesions of the longstanding disease is a problem which will have to be worked out in the future for there is no answer to it now On the other extreme, the longstanding cavitary lesion may progress to involve a whole lobe or a whole lung with a piling up of fibrous

with Langhans giant cells of varying sizes This type of tissue extended for about 3 cm around the wall of the cavity Methenamine silver stains did not reveal any parasites in this tissue Around the borders of the pneumonic area were a scattering of macrophages but no parasites were found in them either See Fig 3c d e Pinkerton and Iverson (9) have described this type of lesion

A few small foci of chronic pneumonia as described by Binford were found but not like his results we were unable to find parasites

There was another unusual case where a large bronchopulmonary node near the hilum was enlarged tremendously but did not result in bronchiectasis There was an invasion of the bronchial mucosa with a chronic granulomatous process but no obstruction of the bronchi resulted At least there was no obvious bronchiectasis and no purulent involvement of the bronchi after the lung had been removed At the time of operation the disease was considered as most likely cancer The pathological diagnosis was tuberculoma Later the parasites of many sizes were found in the large caseous centers which were surrounded by dense bands of fibrous tissue No other lesions were found either in the lungs or in the other lymph nodes Many of the parasites in this lesion of many years duration were found in small nests in which some of the parasites measured no more than a micron in diameter

The pleura is practically always involved in the chronic pulmonary forms of the disease In two cases parasites were found in the pleural fluid In general the pleura resembles that found in tuberculosis with a thick roughened surface above superficial cavities and nearly always adherent to the parietal wall As pointed out by Puckett the round lesions frequently have a heavy smooth button like callous in the pleura which extends down into the fibrotic and caseous lesion Frequently primary tubercles produce a similar effect

No serious attempt has been made or in fact can be made on the limited material at hand to determine the nature of mixed pathology of histoplasmosis and tuberculosis sarcoidosis cancer lymphomas etc Suffice it to say that the few specimens of tuberculosis seemed to present more polymorphonuclear leukocytes than

tissue to great thickness This same result occurs on rare occasions in tuberculosis It takes place in a person who has enough resistance to cause a benign course of the disease yet not enough to bring about complete healing There is one very good example of this which are shown in the illustrations

In the lymph nodes there is seen to be similar variation of lesions to those found in the lungs although up to this time there have been no ruptures of lymph nodes into the bronchi which we have observed or which have been reported in the literature As mentioned in the chapter on pathogenesis the lymph nodes are frequently enlarged to great size causing pressures that result in in flow stasis by pressure on the vena cava and bronchiectasis by pressure on the bronchi We have to date seen five cases where the pressure on bronchi resulted in bronchiectasis The resulting disease in no case was due to the active histoplasmosis but was a result of pressure leading to a secondary infection In one case the parasites were grown from a lesion of only a few years duration but all the others were from an old process where the parasites were found only by staining an old calcified lymph node with an entirely nonspecific disease in the bronchi In the case in which the cultures of H capsulatum were grown it is doubtful if the parasites played any role in the bronchiectasis because only a few parasites were found in the walls of the bronchi but large masses of them were found in the encapsulated and caseous foci (See Fig 6 also Chapter 22)

The granulomatous and chronic pneumonic types of disease are a part of the pathology of histoplasmosis just as is caseation cavitation and calcifications The reasons for the fibrotic changes instead of caseous are not yet evident but there are some observations that might be mentioned as in the illustration where there was a resolving cavity and immediately beneath the cavity was a consolidated mass that appeared like caseous pneumonia of tuberculosis In fact the first time this type of lesion appeared it was called tuberculosis until proven otherwise When a section was made through it however the knife met resistance and the surface left was smooth and rigid On microscopic study there was a network of fibroblasts fibrous tissue epithelioid granulomatous nodules

ROENTGENOGRAPHIC ASPECTS OF HISTOPLASMOSIS

FREDERIC N SILVERMAN

Information derived from radiologic examinations and related to the problem of histoplasmosis was restricted initially to epidemiologic studies of histoplasmin sensitivity and the incidence of intrathoracic calcification —calcification which in general had little to differentiate it from that of tuberculosis The value of these studies lay largely in supporting and emphasizing the fact that all that calcifies is not tuberculosis Subsequently as active proved cases began to be recognized roentgenographic patterns of acute subacute and ultimately chronic disease appeared in the literature These roentgenographic patterns were primarily pulmonary With increasing interest in the disease the clinical and pathological spectrum of the disease became clarified critical comparison of shadows and substance could be made and the details of the pulmonary lesions and the significance of the extrapulmonary lesions became clear

This section will review the roentgen manifestations of histoplasmosis in this historical perspective and sequence Wherever possible the examples will be from proved cases in other instances the criteria for the diagnosis are such as would be acceptable to most students of the subject There will be no attempt to discuss differential diagnosis

For purposes of this presentation the classification to be developed will be primarily morphologic and can be outlined as follows

I Intrathoracic calcification

  A Primary complex
  B Separate components of primary complex
  C Several foci with nodes
  D Disseminated calcification

were usually found in histoplasmosis alone Sarcoidosis silicosis beryllosis and other granulomatous conditions are so rarely found with histoplasmosis that more material must be studied before any thing can be said about the altered pathology

In summary it can be stated that much has been learned about the cellular and gross pathology of histoplasmosis but much is yet to be learned There is a rather constant pattern which the disease manifests in both its progressive type as well as in its varying stages of chronicity There is nothing in either of these fields however that can be considered diagnostic In fact the pathology simulates tuberculosis so closely that it is very easy to mistake the two and in times past most of the round lesions that have been removed have been called tuberculomata The disease also simulates sarcoidosis at times so that they cannot be separated without a search for the parasite

In the last analysis therefore it is mandatory to find the para sites before a diagnosis can be made Fortunately in the majority of active cases cultures of the parasite may be grown and in active and particular caseous and calcified lesions the methenamine silver stain when properly applied will identify the parasites in over 80% of the cases

REFERENCES

1 Darling S T A protozoan general infection producing pseudotubercles in the lungs and focal necroses in the liver spleen and lymph nodes *J A M A* 46 1283 1906

2 Schwarz J General aspects of the pathology of histoplasmosis *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 pp 1? 13

3 Puckett T F Pulmonary histoplasmosis—a study of ?? cases with identification of H capsulatum in resected tissues *Am Rev Tuberc* 67 453 4?? 19?3

4 Zimmermann L F Demonstration of histoplasmosis and coccidioides in so called tuberculomas of lung Preliminary report of thirty five cases *Arch Int Med* 94 690 19?4

5 Binford C H Histoplasmosis Tissue reaction and morphologic variations of the fungus *Am J Clin Path* ?5 19?

6 Vivian D N Weed L A McDonald J R Clagett O T and Hodgson C H *Surg Gynec & Obst* 99 53 62 July 1954

7 Sweany H C Gorelick D F Coller F C and Jones J I Pathologic findings in benign pulmonary histoplasmosis *Tr of 17th Conference on the Chemotherapy of Tuberculosis* Feb 19?8 Memphis Tenn pp 32 3??

8 Sweany H C Gorelick D F Coller F C and Jones J I Pathologic findings in benign pulmonary histoplasmosis *Dis Chest* 34 119 137 and 2?? 275 1958

9 Pinkerton H and Iverson L Histoplasmosis Three fatal cases with disseminated sarcoid like lesions *A M A Arch Int Med* 90 45? 195?

between the incidence of intrathoracic calcification and the incidence of positive skin sensitivity to histoplasmin has been alluded to in the section on epidemiology. Notwithstanding some of the identifying features which will be discussed in subsequent paragraphs, it is necessary to emphasize the fact that intrathoracic calcification is not specific and that skin sensitivity tests are the first step in the evaluation of the nature and significance of intrathoracic calcification demonstrated in a roentgenogram of the chest.

The Calcified Primary Complex

As in pulmonary tuberculosis, histoplasmosis when calcified commonly presents as a primary complex. One finds a calcified focus in the parenchyma of the lung, which by appropriate tangential projection can be shown to occupy the subpleural position indicated in the section on pathology. More frequently the primary calcified focus is found in the routine frontal projection of the chest in the peripheral portions of the lung (Fig. 1). Careful anatomical studies of the type undertaken in tuberculosis by Kuess (1), Ghon and Kudlich (2) and others (3) have not been undertaken in histoplasmosis apart from the relatively small series described by Straub and Schwarz (4), but there is no reason to believe that the distribution of primary foci should be any different. The primary focus should have a chance distribution throughout the lung with some slight tendency to be located in the lower two thirds of the lobes rather than in the apical portions, primarily on the basis of volume of lung available. This is no place to enter into the arguments on the distribution of primary lesions; from the practical standpoint the location of the primary focus is no different from that in primary tuberculosis and has no specific diagnostic significance. The size of the primary focus, however, may have diagnostic information not inherent in the location. Anatomical studies by Straub and Schwarz (4) and radiologic studies by Serviansky and Schwarz (5) have suggested that a parenchymal calcifying focus more than 5 mm in diameter is found appreciably more frequently in association with a positive histoplasmin skin test and in areas where histoplasmosis is endemic than in association with a positive tuberculin test or in areas where histoplasmosis is not endemic.

- II Intrathoracic soft shadows
  - A Infiltrate with hilar nodes
  - B Segmental or lobar consolidation
  - C Lymphadenomegaly
  - D Disseminated soft shadows
  - E Coin lesions—histoplasmomas
  - F Pleural reactions
- III Pulmonary fibrocavitary disease
- IV Extrapulmonary foci
  - A Cervical nodes
  - B Axillary nodes
  - C Splenic calcification
  - D Bone
  - E Other organs
- V Hepatosplenomegaly with normal chest
- VI Normal chest with positive blood and/or bone marrow culture

The subsequent discussion will follow in this sequence

INTRATHORACIC CALCIFICATION

Intrathoracic calcification has long been the hallmark of pulmonary tuberculosis So well implanted is the concept that intrathoracic calcification is prima facie evidence of pulmonary tuberculosis that even today consideration of other diseases generally comes at best is a second choice Nevertheless in certain areas of the country other conditions so frequently exceed tuberculosis as a cause for intrathoracic calcification that its roentgenographic observation should serve merely as an indication that skin sensitivity tests are in order if not to identify the nature of the calcification at least to exclude some of the possibilities particularly pulmonary tuberculosis In the Ohio and Mississippi River basins histoplasmosis is so much more frequently a cause of intrathoracic calcification than is tuberculosis that on statistical grounds alone a physician may earn the reputation of being a diagnostician by favoring the diagnosis of mycologic disease rather than mycobacterial disease in every instance The close correlation



Fig 2 Calcified primary complex with "mulberry" type of calcification in apical focus. Note intrapulmonary node calcification between primary focus and hilar node. Young adult. Histoplasmin positive, tuberculin negative. (Courtesy Am J Roentgenol Rad Therapy & Nuclear Med 1950)

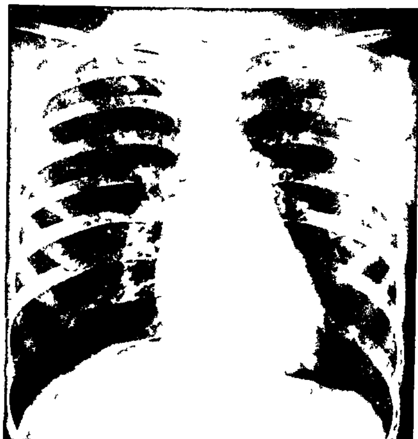


Fig 1 Classical calcified primary complex in proved histoplasmosis *Calcified* node removed at operation for closure of patent ductus arteriosus and H capsulatum found within it (Courtesy *Am J Med* 1955)

The roentgen appearance of the calcification has been suggested (6) as having etiologic significance stippled calcification of the mulberry type (Fig 2) and focal calcification with a soft halo around it (Fig 5 3 and 4) have been said to indicate histoplasmosis more frequently than other diseases Both types of calcification however are found in other diseases and in any given case statistical probabilities serve only to maintain a high index of suspicion diagnosis must be supported by more definitive measures In an area endemic for histoplasmosis any type of calcification is more likely to be associated with a positive histoplasmin skin test than



Fig. 3B Enlargement of area in insert

frequently with positive histoplasmin skin tests than tuberculin tests and to occur with greater frequency in the endemic area for histoplasmosis than in non endemic areas. This radiologic study supported the morphologic investigations of Straub and Schwartz (4). The observation of the large size of the calcific lesions and the extensive distribution had been remarked by Long and Stearns in 1913 (7) prior to any knowledge of the relationship to histoplasmosis. The large size was also a feature in the early report by Furcolow (6) in which the progression of pulmonary infiltrates to calcification was carefully documented.

Calcifications along the lymphatic drainage from the primary focus to the hilum occur with recognizable frequency (Fig. 2) but provide no specific diagnostic information. A pleural reaction is seldom recognized in primary histoplasmosis, even less so than in primary tuberculosis where its frequency is such as to properly permit its inclusion among the components of the primary complex. In one known instance H. capsulatum could be found on direct

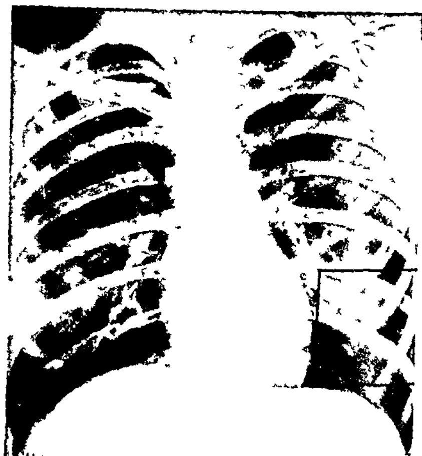


Fig 3A Halo focus Calcific shadow with "soft" density surrounding it Histoplasmin positive tuberculin negative

with a positive tuberculin skin test In general it is wise to consider intrathoracic calcification merely as an expression of disease capable of causing necrosis of tissue but permitting survival and not indicative of any specific disease in itself

Calcification in the hilar lymph nodes draining the parenchymal focus occurs as in tuberculosis (Figs 1 and 2) Here again the size of the lesion may have more diagnostic significance in identifying a given shadow as histoplasmosal than its morphology or the distribution of calcification Serviansky and Schwarz (5) found large lesions (more than 6 mm in diameter) to be associated more

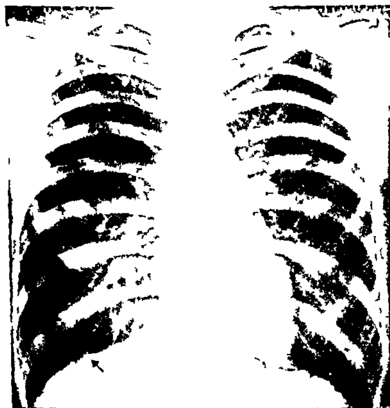


Fig 5A Effect of technical factors on non visualization of components of primary complex Primary focus (*arrow*) clearly seen in LA projection but cardiac density and spine density obliterate calcification in regional lymph nodes (central) draining this area

nation (Fig 5) Failure to demonstrate a parenchymal lesion when a hilar calcification is present may also have technical factors at fault not uncommonly in a routine PA projection what appears to be a hilar focus actually represents a parenchymal focus anteriorly or posteriorly subpleural in the mesial portion of the lung Lateral projections clearly demonstrate the position of this shadow (Fig 6) Other factors which have to do with demonstration of calcium shadows must also be kept in mind these include the size and density of the calcifying focus (Fig 7) Moreover calcium may



Fig 4 Halo focus in apical region

smear of cellular components of a massive pleural effusion (8) Recently a positive culture from pleural fluid was obtained in a fatal case (9)

Separate Components of the Primary Complex

In not every instance of calcifying histoplasmosis is the full complement of calcified shadows recognized Occasionally one may find only a parenchymal lesion at other times only a lesion in the hilum is recognized Failure to identify a hilar lesion may be related to the fact that calcification did not occur in the hilum or more frequently that the technical factors in demonstrating the calcification preclude visualization under standard conditions of examination It is quite probable that the carinal lymph nodes are involved with as great if not greater frequency than are the peribronchial nodes however because of their position behind the heart and superimposed on the bony structure of the vertebral column calcification within them may be missed in routine exami

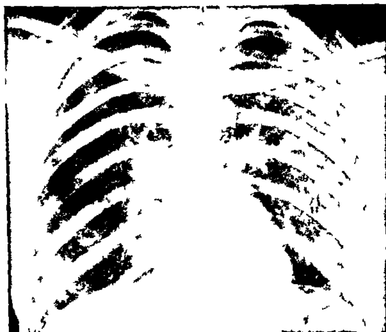


Fig 6A Technical factors in demonstration of components of calcifying histoplasmosal complex Classic calcifying complex in frontal projection

Several Foci with Nodes

As in tuberculosis there may occur several foci of calcification with hilar lymph nodes more marked on or exclusively restricted to one side or the other The multiple parenchymal foci (not disseminated calcification) probably represent several primary foci in different stages of evolution at the time that allergy and necrosis of tissue took place This interpretation is supported by the fact that when three or four primary foci are present in the lung they are appreciably larger than the disseminated fine calcifications noted in the more common form of the disease

Disseminated Calcification

Statistically this variety of intrathoracic calcification (9) is most likely to represent histoplasmosal infection this has been amply proved by the epidemiologic studies of Furcolow *et al* (12)



Fig 5B Effect of technical factors on non visualization of components of primary complex Oblique films shows primary focus but also shows cluster of large calcifying carinal nodes Note bifurcation of trachea outlined by air in lumen above nodes

be lost from a lesion either by resorption as in tuberculosis (11) or by expectoration as a broncholith (10) (Fig 8) A fairly large lesion in infancy may contract and scar down to the point of roentgen invisibility in childhood or later Other possibilities for the absence of a parenchymal lesion would include an endobronchial primary focus as well as a primary lesion healing without calcification

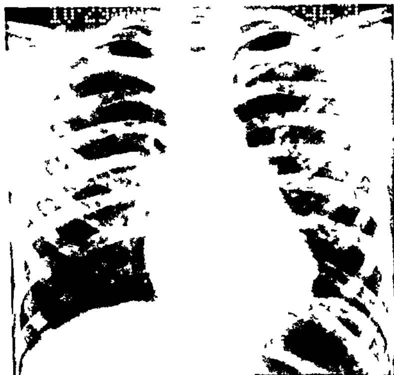


Fig 7A Technical factors in demonstration of histoplasmosal lesion Frontal projection rounded lesion in base of right lung almost completely obscured by diaphragm

shadows represents multiple foci of pneumonitis resulting from the inhalation of a large number of organisms The analogy with hematogenous disseminated tuberculosis however is so great that one may equally strongly support the contention that the disseminated lesions represent hematogenous dissemination The invasion of the blood stream is well known in the disease and may occur in the absence of significant clinical symptoms Although in epidemics the one possible exposure to the organism is commonly known in non epidemic cases it is quite probable that recurrent multiple exposures to the organism take place particularly with individuals living within the endemic area or in environments



Fig 6F Technical factors in demonstration of components of calcifying histoplasmosal complex Same patient lateral projection Two calcifying parenchymal foci without recognizable hilar node calcification

With the extensive application of antituberculous drugs the initial consideration that disseminated calcification can result from healed miliary tuberculosis may now become a reality and the security of etiologic diagnosis may thereby suffer Disseminated calcification not infrequently follows widespread snow storm lesions during the acute phase of the disease (Fig 10) Occasionally it appears without obvious antecedent illness Lehan and his associates (19) have suggested that the pattern of disseminated snow storm



Fig 7C Technical factors in demonstration of histoplasmosal lesion Appropriate right anterior oblique projection shows rounded lesion clearly

spores of Histoplasma capsulatum Their pattern is extremely variable but the following forms occur with sufficient frequency to permit a suggestion of histoplasmosis by the radiologist when the diagnosis is not suspected by the clinician Calcification of the types described above takes place in a high percentage of these soft lesions as healing progresses (see Fig 10) it may make its appearance as short as 5 months after the clinical onset or as long as six years after (13 14) Furcolow (6) states that in the course of following several hundred children with pulmonary infiltrates and positive histoplasmin sensitivity there was little or no tendency for new lesions to appear or for individual lesions to progress



Fig 7B Technical factors in demonstration of histoplasmosal lesion Lateral projection superimposition of lesion on spine also tends to obscure it

heavily contaminated by Histoplasma capsulatum The radiographic manifestations therefore may represent the results of repeated exposures or of a subclinical disease aggravated by superimposed overwhelming exposure

INTRATHORACIC SOFT SHADOWS

These lesions occur as incidental observations or in association with clinical signs and symptoms commonly following known exposure in environments known to contain large numbers of

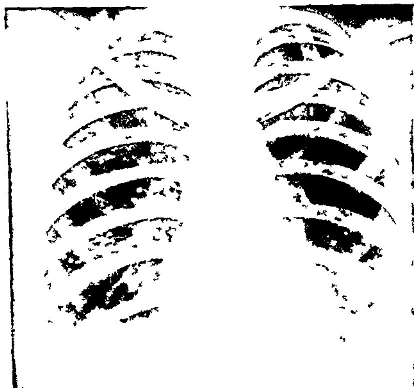


Fig 9 Disseminated calcification Strongest radiographic evidence of previous histoplasmosis (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

Infiltrate with Hilar Nodes

The radiologic picture of acute pulmonary consolidation can occur with histoplasmosis Commonly the infiltrate is a relatively soft ill defined area seldom more than 2 3 cm in diameter which because of the hilar lymphadenomegaly bears a very close resemblance to primary pulmonary tuberculosis (Fig 11) This was one of the first forms of the disease proved to result from infection with Histoplasma capsulatum Not all of these instances heal by calcification but there is a tendency for the infiltrate and the prominent nodes to persist for a longer period of time than in the case of acute



Fig 8A Loss of calcium shadows in chest film due to expectoration of broncholiths August 1956 (Courtesy *Am Rev Tuberc* 1957)

Fig 8B Loss of calcium shadows in chest film due to expectoration of broncholiths October 1956 after expectoration of broncholiths Compare areas above right hilum (Courtesy *Am Rev Tuberc* 1957)

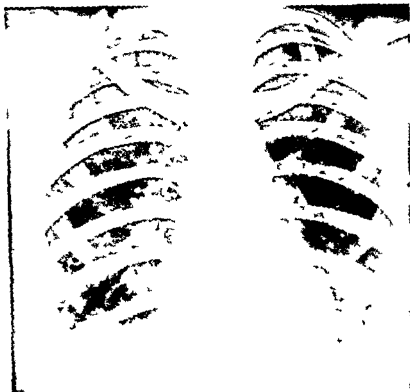


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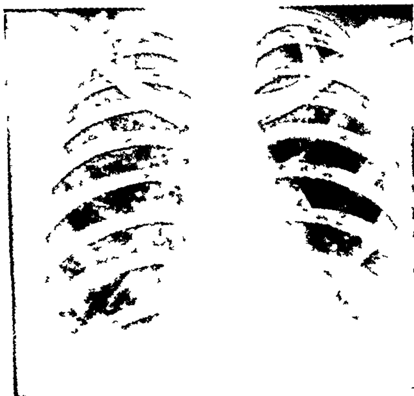


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Fig 10 A Serial films illustrating development of disseminated calcification November 1947 Clinically atypical pneumonia disseminated soft shadows (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

pyogenic pulmonary infection Occasionally the clinical signs may antedate positive roentgen signs by weeks to months In a very broad sense the radiologic manifestations are similar to atypical pneumonia The involvement is usually unilateral and when resolution of the parenchymal focus occurs there may be concurrent diminution of the hilar adenopathy

Segmental and Lobar Consolidation

When the lymph nodes are so placed as to be able to compress a bronchus or when endobronchial reaction is of a sufficient magnitude to permit obstruction of the lumen segmental or lobar consolidation can occur and is radiologically indistinguishable from

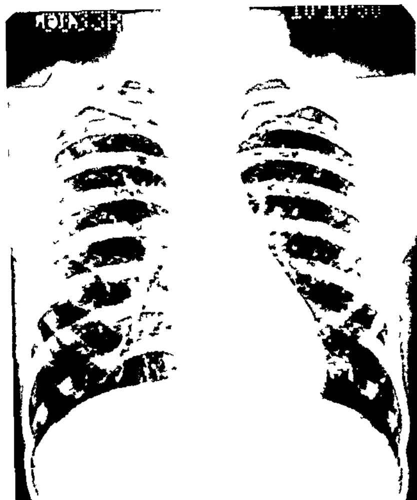


Fig 10B Serial films illustrating development of disseminated calcification October 1950 Earliest calcification seen in annual follow up film (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

that produced by any other cause (Fig 12) Lobar consolidation is less common than is segmental disease As a general rule with coughing up of secretions or with subsidence of the acute reaction in the bronchial tree the apparent consolidation which constitutes in large measure atelectasis tends to clear It is quite probable that

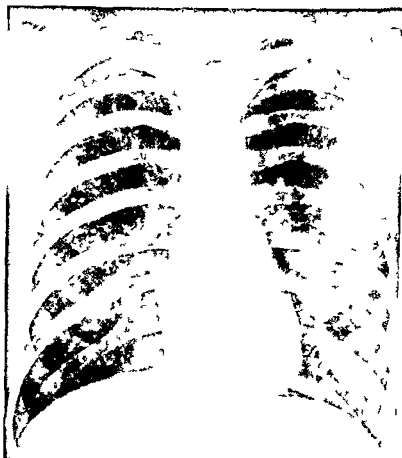


Fig 10C Serial films illustrating development of disseminated calcification February 1958 Well-developed disseminated calcification

this reaction is comparable to that of epituberculosis in primary tuberculous infection Bronchiectasis following bronchial obstruction by *histoplasmosal lymph nodes has been reported (27) but from* analogy with the conditions obtaining in tuberculosis one may suspect that the bronchiectasis unless secondarily infected is very likely reversible However in an endemic area non reversible bronchiectasis may well have had its origin in histoplasmosis

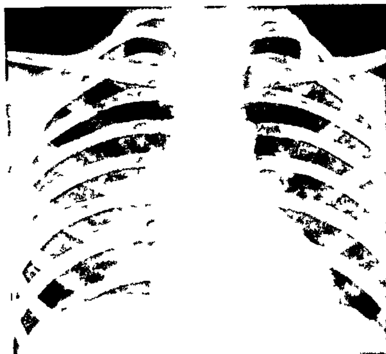


Fig 11 Infiltrate with hilar nodes Note fine interlobar pleural reaction

Lymphadenomegaly

Enlargement of hilar lymph nodes may be the only radiographic manifestation of pulmonary histoplasmosis (Fig 13) This may be spurious if the primary lesion is hidden in the shadow of the lymph nodes and enlarged hilum or if the primary focus should happen to be endobronchial so that the drainage into the lymph nodes is unassociated with a parenchymal lesion The node shadows tend to be quite large persist for months and may produce as mentioned above signs of complete or incomplete obstruction With incomplete obstruction emphysematous changes in the portion of lung supplied by the involved bronchus may be anticipated with complete obstruction atelectasis occurs (Fig 14) Even large lymph nodes may disappear gradually without demonstrating calcification but in general prominent lymph nodes in the hilums can be

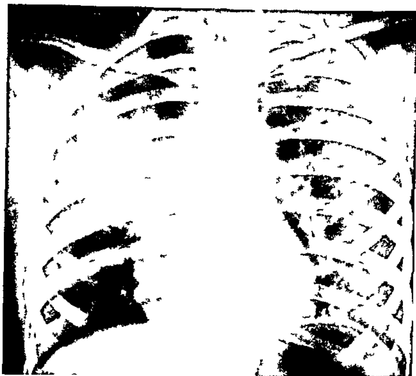


Fig 12A Segmental consolidation with slow resolution January 1957 Acute febrile illness with respiratory symptoms

expected to result in hilar calcification According to Serviansky and Schwarz (5) Furcolow (6) and others this calcification is frequently stippled (Fig 15)

Disseminated Soft Shadows

These are the so-called snow storm shadows which can represent either hematogenous dissemination or the results of dissemination through the bronchial tree of multiple infecting agents (11) The shadows usually tend to be somewhat larger than those seen in hematogenous disseminated tuberculosis and one is frequently surprised by the extensive nature of the shadows radiographically and the absence of clinical signs and symptoms The fine reticulated pattern in the group of cases reported initially by Sabin (15) and Felson *et al* (16) tends to be somewhat unusual inasmuch as

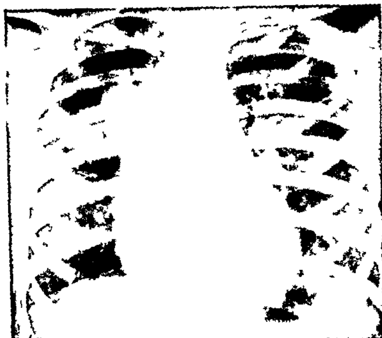


Fig 12B Segmental consolidation with slow resolution August 1937 Clinically greatly improved Note appearance of rounded node above right hilum We would anticipate a large (more than 1 cm) calcification to develop here

calcification has not developed in any of the individuals followed The recent suggestion by Emmons (17) that this epidemic may have been caused by cryptococcosis rather than histoplasmosis if it can be proved may tend to remove this fine reticulated pattern from the group known to be associated with histoplasmosis The disseminated soft densities are too numerous to count (Fig 16) They vary between 1 5 mm in size occasionally there is superimposed localized infiltrate and hilar lymphadenomegaly is commonly recognized The development of calcification is very common in the multiple fine nodular variety it may not be clearly identified for several years and resolution of the surrounding soft density is exceedingly slow Increasing calcification can be observed for years after symptoms have completely disappeared (see Fig 10)



Fig 13 Enlargement of lymph nodes only Two nodes or groups of nodes are recognized one above the right hilum and the other below the left hilum A separate parenchymal lesion could not be identified even on lateral projections.

Coin Lesions—Histoplasmomas

Within the past ten years the introduction of mass chest surveys has brought forth a considerable interest in the nature of so called coin lesions found in usually asymptomatic individuals (see Fig 7) As a general rule many of these have been considered to represent neoplasm and must be considered neoplasm in the appropriate age group Radiologists have attempted to differentiate neoplastic lesions from inflammatory lesions by the presence of calcium within the inflammatory group Sometimes calcification can be demonstrated only by tomography (Fig 17) To Puckett (18) belongs the credit for pointing out that many of these coin

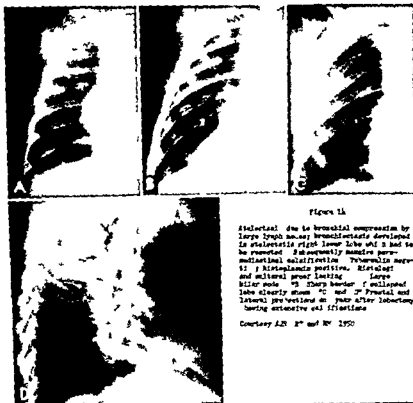


Figure 14

Atelectasis due to bronchial compression by large lymph nodes; bronchiectasis developed in atelectatic right lower lobe which had to be resected. Subsequently massive para-mediastinal calcification. Tuberculin nega-ti[illegible]; histoplasmin positive. Histologic and cultural proof lacking. [illegible] Large hilar node. "B" Sharp border of collapsed lobe clearly shown. "C" and "D" Frontal and lateral projections [illegible] years after lobectomy showing extensive calcifications.

Courtesy A.R. [illegible] and [illegible] 1950

Fig 14

lesions actually represent histoplasmomas inflammatory tumor masses comparable to the tuberculomas which have been known for many years

The presence of a small daughter lesion around the primary focus has been a very helpful point radiologically in identifying inflammatory changes as distinct from neoplastic ones moreover this characteristic appears to be more frequent in histoplasmosis than in tuberculosis Unfortunately the daughter lesions are recognized histologically more easily than radiographically because of technical factors By the same token calcification may be present histologically but be radiologically invisible The fact that these lesions do not increase in size as opposed to neoplastic lesions is a



Fig 15 Stippled calcification in mediastinal lymph node Close up of area of primary complex shown in Figure 1 The irregularity of the calcification and its size are clearly demonstrated

helpful differential point if serial films are available Occasionally with an increase in the density of the lesion possibly due to deposition of calcium which is not yet clearly definable roentgenographically there is a spurious enlargement of the lesion The value of the histoplasmin and tuberculin skin tests in the elucidation of the nature of these lesions is obvious Nevertheless assurance that a given visible shadow is responsible for a positive cutaneous reaction can not be given unequivocally and careful evaluation is indicated in each case

Pleural Reaction

Pleural reactions occur but are infrequent (Fig 18) In one of the fatal cases described by Silverman *et al* (8) a massive pleural

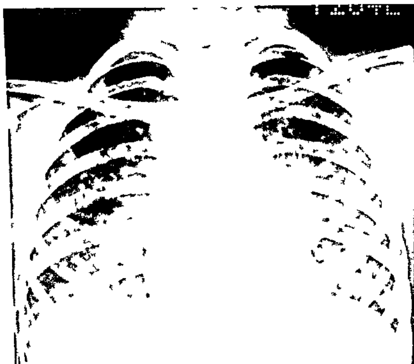


Fig 16A Disseminated soft shadows July 1949 Mild respiratory symptoms (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

effusion was present and from this pleural effusion could be cultured large numbers of Histoplasma capsulatum In fact the organisms were actually found in cells obtained on direct smear of the pleural fluid Massive accumulation of fluid however is uncommon even though the pathological material indicates that the primary focus is so frequently subpleural and that a pleural reaction is present at the site of the lesion Perhaps just as in primary tuberculosis a local pleuritis is the rule rather than the exception but does not reach the level of radiologic demonstration unless a significant allergic reaction is involved (see Fig 11)

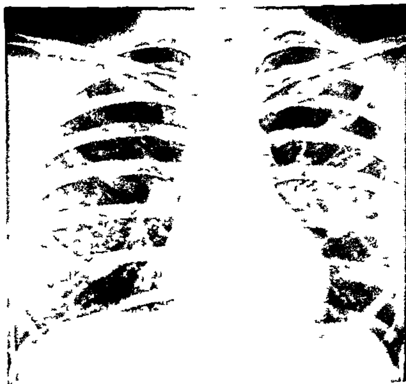


Fig 16B Disseminated soft shadows December 1953 Faint nodularity throughout both lungs (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

PULMONARY FIBROCAVITARY DISEASE

Although histoplasmosis as a cause of intrathoracic calcification has gradually dislodged tuberculosis from its position as the exclusive cause for this roentgenographic phenomenon the presence of cavitary disease in the lung still evokes the diagnosis of tuberculosis with practically no differential diagnosis considered The fact that effective antituberculous drugs and surgical skills are now available for tuberculosis may justify to some extent the perpetuation of this attitude however the proof that pulmonary histoplasmosis may develop into a chronic fibrocavitary type radiologically indistinguishable from tuberculosis merits consideration of a differential diagnosis In fact the chronic fibrocavitary type



Fig 17A Histoplasmoma coin lesion Solitary rounded density in left lower lobe (Courtesy Dr Jan Schwarz *New England J Med* 1958)

of pulmonary histoplasmosis may carry a less favorable prognosis than tuberculosis at the present time and the differentiation may have significant prognostic value (27)

Roentgenographically one recognizes the same features which have been found in tuberculosis irregular areas of consolidation and fibrosis are adjacent to localized radiolucent areas some of



Fig 17B Histoplasmoma coin lesion Tomogram demonstrating calcium densities within lesion (Courtesy Dr Jan Schwarz New England J Med 1958)

which represent loculated emphysema but others of which represent actual destructive cavities communicating with a bronchus through which the destroyed parenchyma has been extruded by expectoration (Fig 19) Apical and subapical locations are the rule beginning as infiltration and progressing to cavitation with surrounding parenchymal disease Spread to other portions of the same lung or even to the opposite lung is not uncommon This too tends to be in the apical region Calcification in nodes is a common associated finding and suggested to Lehan *et al* (19) that cavitary disease may represent a re infection type of histoplasmosis comparable to the reinfection type of tuberculosis



Fig. 18. Pleural reaction in probable histoplasmosis. Cultural proof lacking.

The radiologic features are indistinguishable in the two diseases; however, the organism can be cultured by examination of expectorated material or material obtained on bronchoscopic exploration. Even the knowledge that tuberculosis is present should not exclude consideration of histoplasmosis in such lesions in individuals in or coming from the endemic area. Both diseases have been shown to co-exist, and the fact that radiologic cure or improvement, at least, does not take place following institution of what would be adequate therapy for one disease may be the indication for the search of the other. Fibrocavitary disease of this type is not uncommonly associated with other chronic pulmonary disease, particularly carcinoma (Fig. 20). This is not remarkable, since individuals with chronic fibrocavitary disease tend to be somewhat older than individuals with other forms of pulmonary histoplasmosis. When the "cavities" are produced by loculated emphysema

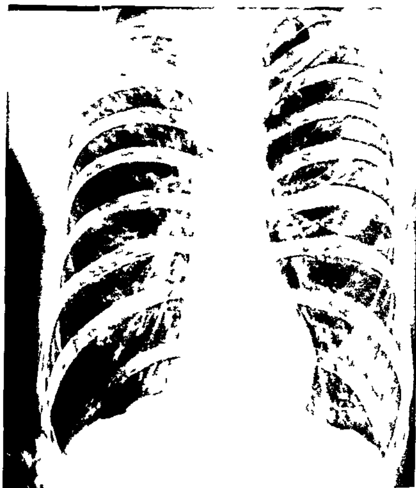


Fig 19 Fibrocavitary pulmonary histoplasmosis Organisms in sputum treated by lobectomy *H capsulatum* also cultured from resected lung tissue and nodes (Courtesy Dr H C Sweany)

rather than by destruction of tissue remarkable recovery can take place in many instances lesions remain stationary over long periods of time Complications of pneumothorax and broncho pleural fistula have been reported (11)



Fig 20 Fibrocavitary pulmonary histoplasmosis complicating carcinoma Both conditions diagnosed ante mortem and proved post mortem (Courtesy *Am J Med* 1955)



Fig 21 Cervical and axillary lymphadenitis in proved subsequently fatal histoplasmosis Organisms obtained from surgically drained cervical nodes

EXTRAPULMONARY FOCI

Extrapulmonary foci of histoplasmosis have been known since some of the earliest descriptions when mucocutaneous junction lesions and destructive processes in the nose and throat suggested that the disease would have considerable interest for otorhinolaryn



Fig 2 Extrapulmonary calcification in axillary lymph node in association with intrapulmonary calcific lesion Histoplasmin positive tuberculin negative (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

gologists (20 21) Although absolute proof is not yet available nevertheless the evidence strongly supports the possibility that these lesions as the cutaneous lesions of blastomycosis (22) represent foci distributed via the blood stream as a complication of the primary disease rather than foci originating in the area where they are observed The statistical study of Schulz (23) disagreed with this interpretation even though the lung was the most frequently involved organ in adults In children hematogenous dissemination was more acceptable statistically In this respect histoplasmosis probably differs somewhat from tuberculosis where extrapulmonary foci have been known to develop by direct infection The lesions of the mucous membranes of the mouth nose and throat have little radiologic significance in themselves On the other



Fig 23A Extrapulmonary calcification in spleen in vivo Chest film in 71 year old female with large characteristic pulmonary calcific focus Splenic calcification barely noticeable (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1956)

hand extrapulmonary foci in lymph nodes where calcification occurs more frequently is of radiologic interest Proved instances of cervical adenitis with calcification resulting from histoplasmosis are not immediately available however the clinical observation of proved disease in the acute stage in these areas would make this



Fig 23B Extrapulmonary calcification in spleen in vivo Heavily penetrated film over splenic area Multiple calcifications clearly shown Compare with

possibility a very real one (Fig 21) In individuals with calcification in cervical lymph nodes and who react to tuberculin and histoplasmin the identification of the cause for the calcification is as insecure as it is when calcification occurs in the lung Calcification has been observed in an axillary lymph node in a child who has a positive histoplasmin skin test and a negative tuberculin test (Fig 22) although this is not proved it strongly favors the possi

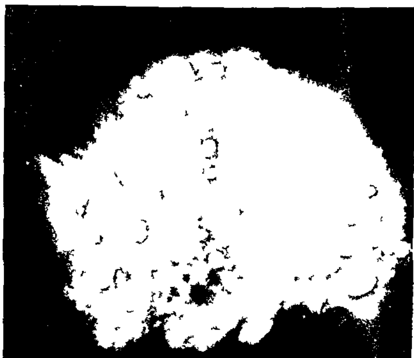


Fig 24A Extrapulmonary calcification in spleen post mortem Note typical rounded concentrically calcified lesions Spleen (Courtesy *New England J Med*)

bility that this calcification did in fact result from disseminated histoplasmosis Whether the dissemination was via the blood stream or via the lymphatics from the lung is open to question Insofar as cervical axillary and even infradiaphragmatic lymph nodes are concerned both pathways are possible However the finding of splenic calcification first pointed out by High (24) almost certainly requires hematogenous dissemination The incidence of splenic calcification has been studied by Schwarz *et al* (25) and corresponds as does the incidence of intrathoracic calcification to the incidence of histoplasmin sensitivity In addition the frequency of splenic calcification differs in the endemic area in comparison with areas where histoplasmosis is not endemic Characteristic features of such calcifications as opposed to calcifications which are found in up to 25% of spleens in non endemic areas



Fig 24B Extrapulmonary calcification in spleen post mortem Note typical rounded concentrically calcified lesions Histologic substrate of lamellated calcified splenic foci (Courtesy *New England J Med*)

include (1) multiple lesions and (2) lesions which are discretely rounded uniform in density or possessing a lamellated appearance which corresponds closely to the histologic findings (Fig 24) Many more involved spleens are found if organs are examined after removal from the body than are found in routine examination because of the effect of filtration of the surrounding tissues on the frequently relatively faint calcific shadows (Fig 24) The demonstration of the frequency of disseminated calcification in the spleen is an important point in clarifying the pathogenesis of histoplasmosis It is truly remarkable that a disease the etiologic agent of which can be spread in such numbers through the blood stream and which can when localized in the spleen cause multiple areas of necrosis it is remarkable that such a disease can occur in so benign a form as to be clinically unappreciated or undifferentiated

from the common upper or lower respiratory infections experienced in the course of normal activity. Disseminated splenic calcifications also suggest that calcific lesions should be found in other organs if an adequate search is made. In fact, lesions in the liver are now known to be present when search is made for them. Strangely enough, the kidney appears to be relatively spared radiologically, but pathologically, at least, this organ is involved (23). The distribution of lesions in the various organs has been described by Schulz (23); however, the majority of these lesions are radiologically invisible.

Adrenal lesions are found with considerable frequency and comment has already been made in the section on clinical manifestations concerning the presence of Addison's disease as a manifestation of histoplasmosis. Adrenal calcification as a direct result of histoplasmosis has not been recognized roentgenographically; even if calcification were found, one probably could not clearly differentiate it from that which tends to occur normally in a certain percentage of individuals, usually children, probably as a sequel to involution of the fetal cortex. It is interesting, too, that notwithstanding the frequency of bone marrow involvement, actual destructive or productive changes in bone have been described only once (26). In this instance there was merely spotty bone atrophy associated with soft tissue thickening about the knee and fluid in the joint. Granulation tissue with cells containing parasitized organisms were found to be invading the bone in the area where the demineralization was noted.

Anatomical lesions in the bowel have been described at post mortem examination and prominent ulcerations have been found associated with massive bleeding; in none of these instances have contrast studies of the bowel been undertaken. It is quite possible that either the granulomatous lesions or the ulcerations could be shown by appropriate contrast examination of the intestinal tract, but identification of any pathologic change as histoplasmosal would not be acceptable on roentgen grounds alone. From the standpoint of lesions which have not yet been found, it is probably pertinent to emphasize that intracranial calcification as a manifestation of histoplasmosis has yet to be proved; this is an important considera-

tion inasmuch as in the endemic area there is a tendency to explain any type of calcification in association with a positive histoplasmin skin test as produced by histoplasmosis This is a dangerous attitude particularly with respect to calcification involving the central nervous system Pathologically lesions in the central nervous system have been found but no calcification has been described as yet

HEPATOSPLENOMEGALY WITH NORMAL CHEST

In the diagnosis of histoplasmosis in infants the finding of hepatosplenomegaly may warrant serious consideration of the disease in question Not infrequently an infant with hematogenous disseminated histoplasmosis fails to demonstrate intrathoracic lesions which would point to the disease having a pulmonary origin but instead does show the massive hepatosplenomegaly which is non specific (Fig 25) In some instances the clinical suspicion of leukemia is raised because of extensive bone marrow involvement the absence of skeletal lesions which occur with considerable frequency in leukemia and the presence of hepatosplenomegaly may be the indication that we are dealing with histoplasmosis instead In fact in one instance known to the author the diagnosis of leukemia was thought to be supported by the fact that a sibling had previously died of leukemia The infrequency of familial leukemia however raised the question of the accuracy of the initial diagnosis and suggested that both children were suffering from disseminated histoplasmosis instead This proved to be the case Perhaps if more of the infants with hepatosplenomegaly were to survive the tell tale shadows would make their appearance in the lung In one case in which death occurred shortly after the diagnosis was made roentgenograms of the chest demonstrated no intrathoracic lesions even though on pathologic examination two well developed primary foci each measuring mm in diameter and containing enough calcium to be gritty when sectioned were found at autopsy (8) These lesions could not be identified even on review of the films when their position was known

from the common upper or lower respiratory infections experienced in the course of normal activity. Disseminated splenic calcifications also suggest that calcific lesions should be found in other organs if an adequate search is made. In fact, lesions in the liver are now known to be present when search is made for them. Strangely enough, the kidney appears to be relatively spared radiologically but pathologically at least this organ is involved (23). The distribution of lesions in the various organs has been described by Schulz (23); however, the majority of these lesions are radiologically invisible.

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pathologic change Not infrequently these individuals are admitted to the hospital for febrile illnesses of an ill-defined nature Laboratory studies are initially negative and routine examinations of the chest and abdomen are unrewarding Recovery or improvement appears to be spontaneous and the patient returns home Subsequently blood cultures taken during the period of acute illness turn out to be positive and the patient is recalled to the hospital for evaluation having no signs or symptoms of his disease or relatively mild illness which can be attributed to histoplasmosis only by inference It is quite probable that this situation obtains in the great majority of individuals who develop asymptomatic or mildly symptomatic histoplasmosis and a positive histoplasmin skin test The appearance of intrathoracic calcification subsequently in these individuals is at times a surprising finding in the absence of obvious soft lesions However it is important to recognize that disseminated disease may be present in the absence of any radiologic signs even more frequently than in the absence of clinical signs and symptoms

SUMMARY

To summarize the radiologic findings in histoplasmosis it may be said that a spectrum of disease exists much as it does in the clinical form the acute forms of the disease if they do have radiologic manifestations are related in large part to the distribution of the organism in the various organs and the susceptibility of the organs and their lesions to radiologic demonstration In the chronic form of the disease the lungs are almost exclusively involved and these demonstrate fibrocavitary changes indistinguishable from those of chronic pulmonary tuberculosis or other chronic pulmonary inflammatory disease In the healed or healing stages calcification takes place with considerable frequency the most frequent lesions are found in the lungs but extrapulmonary foci may make their appearance in the healing phase when they were radiologically invisible during their acute stage Thus the roentgen examination may serve not only as a case finding tool but may also mirror to a greater or lesser degree the extent and severity of the disease

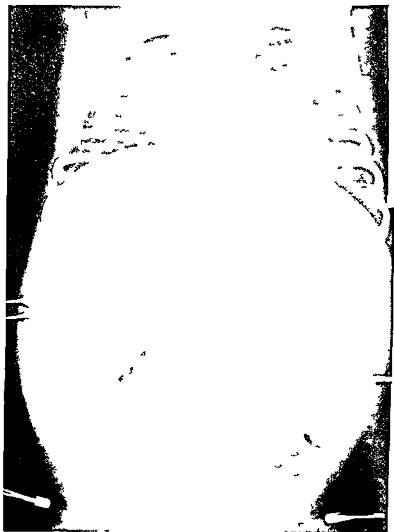


Fig 25 Massive hepatosplenomegaly and normal chest in infant with symptomatic non fatal histoplasmosis (Courtesy *Am J Roentgenol Rad Therapy & Nuclear Med* 1950)

NORMAL CHEST WITH POSITIVE BLOOD AND/OR BONE MARROW CULTURE

In a not insignificant number of patients the diagnosis of histoplasmosis has been proved by positive blood and/or bone marrow cultures in the absence of any radiologically demonstrable

21 Moore M and Jorstad L H Histoplasmosis and its importance to otorhinolaryngologists a review with report of a new case *Ann Otol Rhin & Laryng* *5* 9 1943

22 Schwarz J and Baum G L Blastomycosis *Am J Clin Path* *21* 999 1951

23 Schulz D M Histoplasmosis a statistical morphologic study *Am J Clin Path* *4* 11 ?6 19 1

24 High R H Calcifications in spleen occurrence of histoplasmin and tuberculin reactors *Pub Health Rep* *61* 1 82 86 1946

25 Schwarz, J Silverman F N Adriano S M Straub M., and Levine S The relation of splenic calcification to histoplasmosis *New England J Med* *252* 887 891 1955

26 Key J A and Large A M Histoplasmosis of knee *J Bone & Joint Surg* *24* 281 1942

27 Baum G L and Schwarz J Pulmonary histoplasmosis. *New England J Med.,* *58* 6 7-684 1958

REFERENCES

1 Kuess G De l heredite parasitaire de la tuberculose humaine These de Paris No 210 1898 (Paris Asselin et Houzeau)

2 Ghon A and Kudlich H Die Eintrittspforten der Infektion von Standpunkt der pathologischen Anatomie in Engel Pirquet *Handbuch der Kindertuberculose* Leipzig Thieme 1930

3 Schwarz J Tuberculosis infantil *Rev Chilena Pediat 11* 893 910 1944

4 Straub M and Schwarz J The healed primary complex in histoplasmosis *Am J Clin Path 25* 727 1955

5 Serviansky B and Schwarz J Calcified intrathoracic lesions caused by histoplasmosis and tuberculosis *Am J Roentgenol 77* 1034 1041 1957

6 Furcolow M Development of calcification in pulmonary lesions associated with sensitivity to histoplasmin *Pub Health Rep 64* 1363 1393 1949

7 Long E R and Stearns W H Physical examination at induction standards with respect to tuberculosis and their application as illustrated by review of 53 400 x ray films of men in the Army of the United States *Radiology 41* 144 150 1943

8 Silverman F N Schwarz J Lahey M E and Carson R P Histoplasmosis *Am J Med 19* 410 459 1955

9 Schwarz J Personal communication

10 Baum G L Bernstein J L and Schwarz J Broncholithiasis produced by histoplasmosis *Am Rev Tuberc & Pulm Dis 77* 162 167 1958

11 Silverman F N Pulmonary calcification—tuberculosis? histoplasmosis? *Am J Roentgenol 64* 74 ,64 1950

12 Furcolow M L High R H and Allen M F Some epidemiological aspects of sensitivity to histoplasmin and tuberculin *Pub Health Rep 61* 113° 1144 1946

13 Kunstadter R H Whitcomb F C and Milzer A Primary histoplasmosis with recovery of H capsulatum from the blood and bronchial secretions *J Lab & Clin Med 34* 1290 1949

14 Bronson S M and Schwarz J Roentgenographic patterns in histoplasmosis *Am Rev Tuberc & Pulm Dis ,6* 173 194 1957

15 Sabin A B Miliary granulomatous pneumonitis in a group of men exposed to pigeon excreta *Nat Tuberc A Tr* 1951

16 Felson B Jones G F and Ulrich R P Roentgenologic aspects of diffuse miliary granulomatous pneumonitis of unknown etiology *Am J Roentgenol 64* 740 1950

17 Emmons C W *Proc of Seminar on Histoplasmosis* Jewish Hosp A Cincinnati Ohio Feb 11 1958

18 Puckett T F Pulmonary histoplasmosis A study of twenty two cases with identification of H capsulatum in resected lesions *Am Rev Tuberc 67* 453-476 1953

19 Lehan P H Brasher C A Larsh H W and Furcolow M L Evaluation of clinical aids to the diagnosis of chronic progressive cavitary histoplasmosis *Am Rev Tuberc & Pulm Dis 75* 938 948 19 7

20 Curtis A C and Grekin J N Histoplasmosis a review of the cutaneous and adjacent mucous membrane manifestations with a report of 3 cases *JAMA 134* 1217 194,

tion than one would customarily assume in classifying the clinical types of any disease but is a vital concept in understanding the frequency of clinical disease versus the frequency of infection The actual spectrum of infection or disease accompanying infection has been demonstrated in only a very few diseases such as certain enteric infections poliomyelitis and some other CNS viral infections Perhaps one of the best demonstrations of the spectrum of a fungus disease was that of Dr Smith and his group who studied the clinical spectrum of coccidioidomycosis in Army personnel in the Southwest during World War II (1) The basic problem of the clinical spectrum of illness was brought out by these studies where it was shown that approximately 76% of the patients who developed infection as evidenced by change of skin tests from negative to positive had such a mild infection as to be classified as either asymptomatic or so mild as to be unrecognized Since similar problems exist with tuberculosis and with histoplasmosis it appears desirable to discuss the broad spectrum of response of the host to infection rather than simply clinical illness The proposed classification is considered tentative at best This is especially so since although the disease was only recognized as a widespread problem in 1945 it has now been estimated that more than 30 000 000 people are infected in the United States The clinical concepts of the disease are changing and additional knowledge is being added each year Attempts at the clinical classification of the disease have become increasingly frequent and it is interesting that although 7 references to clinical classifications were referred to at the time of the previous classification in 1956 (2) at least as many clinical classifications have been published in the last two years (3 10)

The basic difficulty with the easy recognition of the disease is explained by the fact that the disease spectrum runs all the way from asymptomatic or mild infection to acute fulminating disease to chronic cavitary disease of long duration Since histoplasmosis is apparently not a new disease it is obvious that the clinical symptoms must mimic other diseases otherwise the characteristic clinical picture would long ago have been recognized and described

Against a background of this mimicry of other well known in

CLINICAL TYPES OF HISTOPLASMOSIS

Michael L Furcolow

With histoplasmosis as with every other infectious disease the disease picture and clinical illness is essentially the result of a battle between host resistance and virulence of the organism Thus the organism may enter the lungs and set up a minor pathological response skin test sensitivity may develop and the patient may have no illness or minimal illness In other cases the pulmonary proliferation of the organism is more extensive and organisms may enter the hilar lymph nodes and not infrequently the circulation In most of these cases also the clinical response is mild and the resulting development of resistance in the individual localizes the organism and overcomes the disseminated foci In still other cases the disease progresses locally resulting in the development of local nodules and even cavities usually at the site of the original pneumonic infiltrations Cavities are not as common as in coccidioidomycosis In still other individuals the growth of the fungus seems unimpeded and progresses through the lungs to the regional lymph nodes and the blood stream and the result is disseminated disease often times with a minimal of pulmonary infiltration Here the organism is found in all the tissues of the body and the prognosis is immediately much more guarded These disseminated cases may be roughly classified into two types the acute type in which the prognosis is decided within the first 1 to 6 weeks and quite often ends fatally and the chronic variety in which the balance is more evenly drawn between the host and the organism and the patient may go on with chronic disseminated disease for a period of a year or two However in these cases also the outlook is grim and most of them eventually succumb to the infection

Any discussion of the clinical types of histoplasmosis must in the opinion of the author include a discussion of the entire spectrum covering the responses of the host to implantation of the fungus Histoplasma capsulatum This is perhaps a broader defini

TABLE I

Cl l Type	S b las Sy ym	Sympt m	T mp t e	Ch t X y	Ag	Cl l F quency	D tlo	R l pse	Labor tory			Pr gnosis
									Skl T t	Ser l gy	Cultu e	
A t p lm ry	A A ymp t m t	\ e	?	Up to 1/3 m st h l	A y age	R re	?	?	+	?	?	Good
	B Symp o m 1 M ld	M l se Fe	Up t 100 101	At lea t 1/3 h les na /3 Nod l r 1/4 P mo ic 1/10 Nodes	A y ge	Prob bly ry fre q t	1 4 d ys	?	+	±	?	Good
	M d ly se	M l se F Co gh	Up t 104 105	Atyp cal p o	A y age	Prob bly f eq nt	5 15 d ys	Common acute phase M y occur l ter	+	+	Prob bly pos ti e ly	Cood
	3 Se (t epi d m)	F r Mal se Ches p C gh	Up to 106	Dissem t d p m t s bo h l gs	You g d lt W kmen Sold rs	Fai ly fre q t	Up to 8 m th	M y occ r	+	+	Pos ti e ly	Good lmost f tal
Chro c p lm ry	P gr (C t ry)	Co gh Sp um M l F	M ld Up t 100 101	F bro C t lly Atyp l Oft n l fi d les	Mostly er 50	M y be p to 10 pe t TB toria	1 10 y rs	U al	+	+	+	Poo ent lly f t l
A te d sem t d	A B gn	Sp k g t m pera H p t megaly Spl m galy a d D rrhea	Up t 106	M y be nega ti	Old h ldre	U k ow	1 8 week	U al	±	+	+	Good
	B P ogres	Sp k g t m p t re H p t m galy Spl m galy d D rrhea	Up t 106	M y be ga t	E peci lly ry yo g d ld w h o her d se se	U k own often di g sed t a t p y	1 6 weeks	?	?	?	?	Always f tal
Chro d sem t d	M coc ta eo	Ulc rs to g l rynx mo th	N e t t rt l t r septic	M y be g t	Old p rso	U k wn	6 week t 3 years		±	+	+	Poor u ually f tal

fections and clinical entities, an attempt will be made to describe the clinical spectrum of histoplasmosis The classification might be divided on what one might call purely clinical grounds stressing mainly the symptomatic types that might be encountered by the physician or it might be done on what could be called more anatomical grounds stressing the organs which are most likely involved and the symptoms which go with such involvement In a prior classification attempted by the author in 1956 the purely clinical approach was used but increasing recognition of the pathogenesis of the disease and its mimicry of tuberculosis has suggested that perhaps it would be better to classify the disease more from an anatomical approach Accordingly the broad classification will be divided into pulmonary histoplasmosis and disseminated histoplasmosis each subdivided into acute and chronic varieties *Localized* lesions and some of the less well classified types of infection will be discussed in the subsequent sections This classification is illustrated in Table I

The division into pulmonary and disseminated may well be criticized since the current concepts of infection (see epidemiology chapter) indicate that practically all infections take place by way of the lungs In other words the organisms are inhaled and regardless of the subsequent clinical picture the primary infection occurs *in the lungs However it does appear worthwhile to divide the* manifestations into primarily pulmonary and primarily systemic or disseminated It should be stated here that the term disseminated as applied in mycology (following the usage of Dr Charles E Smith) *has come to mean the occurrence of lesions or spread of infection* outside of the lungs themselves In other words the term disseminated is not employed in discussing intrapulmonary disease This is a necessary qualification in view of the different application of this terminology in tuberculosis and some other diseases

ACUTE PULMONARY HISTOPLASMOSIS

Asymptomatic Asymptomatic infection is by far the most common type In the reports of the Smith studies referred to earlier on coccidioidomycosis 60% of the patients denied any illness whatsoever (1) It is interesting to note that among laboratory *personnel*

TABLE I

Cl l Type	S b lass Sy ym	Sympt m	T mper l	Ch t X y	Ag	Cli al Freq cy	Du ti	R l p	Labo t ry: Sk T t	Ser l gy	C lture	Pr gn sis
A te p l ry	A A symp t m	\	?	Up to 1/3 m t h l s	A y age	R re	?	?	+	?	?	Good
	B Sympt m 1 M ld	M l ise F r	Up t 100 101	At le t 1/3 h e lesi /3 Nod l r 1/4 P umo ic 1/10 Nodes	A y ge	Prob bly ry fre q t	1 4 d ys	?	+	±	?	Good
	M d ly se	M l ise F Co gh	L p t 104 105	Atyp cal p m	A y ge	Prob bly f eq t	5 15 d y	Common in acut phase M y occur l ter	+	+	P obably positi e early	Cood
	3 S e (t epi d)	F M l Ch t p n C gh	Up t 106	D sem t d p m t both l g	Y g d lt W km Sold rs	F ly fr q ent	Up to 8 m th	M y occu	+	+	Pos ti e rly	Good lmost ne r fatal
Chro c p lm ary	P g (C t s)	Co gh Sp t m M l se F	M ld Up o 100 101	F bros C t lly Atyp l Oft cal f d les	Mostly 50	M y be p t 10 pe t TB san toria	1 10 y rs	L l	+	+	+	Poo ent lly f t l
A t d sem ed	A B gn	Sp k g t m p rat H p t megaly Spl om galy d D rrh	Up to 106	M y be n ga t	Old h ldre	U k w	1 8 weeks	U ual	±	+	+	Good
	B P g es e	Sp k g t m p t e H p tom g ly Spl m g ly d D rr) ea	Up t 106	M y be g t	E peci lly ry y g d ld w h o her d se se	U k own oft di g osed t t p y	1 6 weeks	?	?	?	?	Alw ys f tal
Chro d sem n t d	M coc t eo	Ulcera o gu l rynx mo th	N e t start late sept c	M y be g t	Old r p rso	U k w	6 week to 3 y rs		±	+	+	Poo ally f tal

fections and clinical entities, an attempt will be made to describe the clinical spectrum of histoplasmosis The classification might be divided on what one might call purely clinical grounds stressing mainly the symptomatic types that might be encountered by the physician or it might be done on what could be called more anatomical grounds stressing the organs which are most likely involved and the symptoms which go with such involvement In a prior classification attempted by the author in 1956 the purely clinical approach was used but increasing recognition of the pathogenesis of the disease and its mimicry of tuberculosis has suggested that perhaps it would be better to classify the disease more from an anatomical approach Accordingly the broad classification will be divided into pulmonary histoplasmosis and disseminated histoplasmosis each subdivided into acute and chronic varieties Localized lesions and some of the less well classified types of infection will be discussed in the subsequent sections This classification is illustrated in Table I

The division into pulmonary and disseminated may well be criticized since the current concepts of infection (see epidemiology chapter) indicate that practically all infections take place by way of the lungs In other words the organisms are inhaled and regardless of the subsequent clinical picture the primary infection occurs in the lungs However it does appear worthwhile to divide the manifestations into primarily pulmonary and primarily systemic or disseminated It should be stated here that the term disseminated as applied in mycology (following the usage of Dr Charles E Smith) has come to mean the occurrence of lesions or spread of infection outside of the lungs themselves In other words the term disseminated is not employed in discussing intrapulmonary disease This is a necessary qualification in view of the different application of this terminology in tuberculosis and some other diseases

ACUTE PULMONARY HISTOPLASMOSIS

Asymptomatic Asymptomatic infection is by far the most common type In the reports of the Smith studies referred to earlier on coccidioidomycosis 60% of the patients denied any illness whatsoever (1) *It is interesting to note that among laboratory personnel*

of three years (12) Approximately 30% of these persons developed definite x ray lesions during the six months following conversion Significant numbers of lesions also first became apparent at later intervals following the conversion probably associated with hardening or calcification of the lesions Table II shows the data on which these figures are based

TABLE II

NUMBER AND PER CENT OF HISTOPLASMIN SKIN TEST CONVERTERS WITH PREVIOUSLY NEGATIVE CHEST X RAYS WHO DEVELOPED NEW LUNG LESIONS ARRANGED BY SIX MONTH PERIODS FOLLOWING THE E[illegible]TED DATE OF CONVERSION

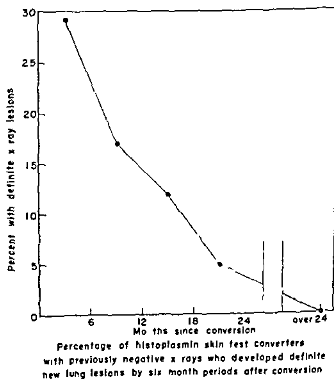
Certainty of Lesions	*Total Number of Persons*	*Months after Conversion* 0 5	6 11	1[illegible] 1[illegible]	18 [illegible]	Over 24
		Number				
Definite	[illegible]	10		4	1	0
Probable	8	3	2		0	0
Questionable	[illegible]		1	1	1	1
Negative	[illegible]	3	31	2[illegible]	17	16
Total	[illegible]	[illegible]	41	33	19	17
		Per Cent				
Definite	3	[illegible]9	1	12	5	0
Probable	10			[illegible]	0	0
Questionable	7	4	[illegible]	3	[illegible]	6
Negative	48	6	[illegible]	[illegible]	90	94
Total	100	100	100	100	100	100

One probable x ray lesion appear 18 months before skin test conversion

Fig 2 shows the frequency of positive complement fixation tests to histoplasmosis among the same group by the months after conversion of the skin tests This figure is based on the results of a total of 219 serological tests on 77 histoplasmin skin test converters It is thus apparent that one fourth to one third of the converters develop either skin lesions or x ray changes or both even though no serious clinical illness could be elicited by questioning Indeed

fairly carefully studied in the Kansas City Field Station (11) who were infected in the course of their work approximately 60% admitted no illness However since some of the illnesses described below will be extremely mild it is quite obvious that they could have been missed Even among these asymptomatic patients x ray lesions are not at all unusual Since approximately one third of the persons with positive skin tests show calcified lesions discernible in their x ray films it is evident that at least this number must develop x ray changes of a significant character during their infection The prognosis in asymptomatic infection is good since if estimates of 30 000 000 people infected in the United States are correct it is obvious that most of them must recover without serious illness or disease

Fig 1 shows the development of x ray lesions among a group of skin test converters followed in a school survey during a period



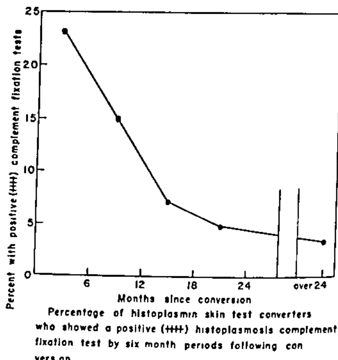
Percentage of histoplasmin skin test converters with previously negative x rays who developed definite new lung lesions by six month periods after conversion

Fig 1

or two With more severe infection similar symptoms are more prolonged there is more of a tendency for a cough to develop and relapses may occur if the patient is ambulated too early In addition the patient usually feels prostrated for a considerable time after recovery Thrombophlebitis sometimes with embolic pneumonia may occur during the acute phase The x ray lesions in the milder type range from a nodule or two in the lungs with or without hilar adenopathy to increasing numbers of lesions usually with hilar adenopathy (see Figs 3 to 6) The lesions are usually of a nodular type although pneumonic infiltrates are seen In the more severe disease a picture resembling atypical pneumonia is frequently seen with infiltration fanning out from both hilar areas



Fig 3 W F age 1 Nodule with calcific center seen in left third interspace with enlarged hilar nodes Serological tests for histoplasmosis positive No history of illne (Courtesy of Public Health Reports)



Percentage of histoplasmin skin test converters who showed a positive (++++) histoplasmosis complement fixation test by six month periods following conversion

Fig 2

the only history of illness obtainable was of the usual colds influenza etc so common among school children Some of these children may therefore have been symptomatic infections if one could ascertain the exact time of infection

Symptomatic Infections The clinical findings accompanying Histoplasma capsulatum invasion of the host may be divided into mild moderately severe and severe or they simply may be classified as being of varying severity running from extremely mild influenza lasting for a day or two to extremely severe non specific illness resembling atypical pneumonia and lasting sometimes for months One suspects that the degree of illness which appears to correlate quite well with the degree of pneumonic infiltration may simply be an expression of the severity of infection or the number of organisms inhaled In the milder varieties the influenza like illness consisting of malaise and mild fever may last only for a day



Fig 4B Healing of the lesion by calcification 5 years later (Reprinted by permission from Rabin Coleman B *et al* *Roentgenology of the Chest* Springfield Ill Charles C Thomas Publisher 1958)

that one can more freely classify together the various symptomatic types of pulmonary histoplasmosis is that epidemiological evidence suggests that perhaps most if not all of the Histoplasma infections are indeed epidemics in miniature there being evidence that most infections even among city children are acquired by visits to point sources and the acquisition of the infection at these point sources (13 also see Epidemiology Chapter)

The epidemics themselves are dramatic instances which in the past have been reported under such interesting names as acute interstitial pneumonitis (14) cave sicknes (15) bat disease (16) Tingo María fever (17) speleologists disease (18) etc



Fig 4A W F age 9 Single pneumonic lesion with nodes in the right midlung noted in April 1948 Tuberculin negative Serological tests for histoplasmosis were originally negative later positive with drop again to negative on recovery No specific history of illness during this time was obtainable Patient attended school regularly (Reprinted by permission from Rabin Coleman B *et al* *Roentgenology of the Chest* Springfield Ill Charles C Thomas Publisher 1958)

This type is accompanied by illness more severe than the isolated pulmonary nodules probably because more lung tissue is involved The most severe type of symptomatic pulmonary infection is the epidemic type of disease where multiple infiltrates are scattered throughout all the lung fields and patients are often severely ill requiring hospitalization for periods of weeks or months With symptomatic histoplasmosis the onset is usually acute becoming more clearly evident with the epidemic type of disease The reason

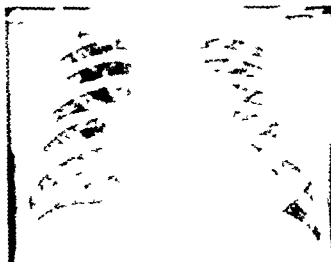


Fig 7A W M age 6 Film of October 4 1950 revealed multiple soft pneumonic infiltrates with perihilar infiltration especially evident in right lung Tuberculin skin tests negative *histoplasmin positive* (Reprinted by permission from Rabin Coleman B *et al Roentgenology of the Chest* Springfield Ill Charles C Thomas Publisher 1958)

It appears that most cases reported as healed miliary tuberculosis are in fact healed miliary histoplasmosis

The physician is often struck by the variation in clinical illness in a single epidemic (20) and by the variety of x ray lesions seen from a very few to what appears to be involvement of most of both lung fields (21) It is on this basis that the postulate is made that most histoplasmosis is contracted at point sources and that the various clinical manifestations depend to a large extent on the degree of resistance of the host and the number of organisms inhaled

For some reason it appears that most of the epidemics have concerned young adults workmen soldiers and persons of that type Whether young previously uninfected adults suffer a more severe clinical response to the disease than persons of younger ages has not been determined However it is clear that among family epidemics the most severe disease tends to occur among the young



Fig 5 W M age 6 Greatly enlarged mediastinal node on right with small infiltration right second interspace Child became ill with colds about 1 month ago with fever and mild cough Symptoms persisted for 6 weeks Tuberculin negative histoplasmin positive serological tests for histoplasmosis strongly positive

Fig 6 W M age 13 X ray November 4 1946 Marked perihilar infiltration bilaterally with atelectasis of right lower lobe Patchy infiltrates in left second and third and right midlung filled Subsequently required lobectomy of middle lobe Cultures of resected lobe negative Histoplasmin positive tuberculin negative serological tests for histoplasmosis repeatedly positive

Indeed one recent author has gone so far as to suggest that perhaps the curse that accompanied the opening of the Egyptian tombs (the Curse of King Tut) might well have been histoplasmosis (19) The epidemics are indeed striking occurrences since the acute onset the coincidental exposure of a number of persons and the severity of the disease usually lead the physician to suspect the point source prior to the actual demonstration of the etiology The wide spread pulmonary infiltrations (Fig 7A) are also now known to be characteristic of this type of disease and indeed are the only type of x ray changes in histoplasmosis which can be regarded as character istic This type of pulmonary change results after the passage of time in most cases in the development of healed miliary calcification of a type formerly called healed miliary tuberculosis (Fig 7B)

CHRONIC PULMONARY HISTOPLASMOSIS

(Progressive Cavitary)

This disease is of extreme interest since at the time of the previous report in 1956 only 15 cases had been reported in the literature and the problem was just developing It is now quite obvious that this problem is of extreme importance in tuberculosis sanatoria throughout the whole country (21) Surveys have been conducted in 37 sanatoria in 24 states A total of almost 20 000 sera have been tested and more than 1 000 positive serological tests have been found The frequency of positive serological tests increases with the frequency of histoplasmin sensitivity in the area varying from 2% in areas of low sensitivity to more than 7% in areas of high histoplasmin sensitivity Since more than one third of these cases can be demonstrated to be active disease by careful cultural studies it is quite obvious that this is a matter of great significance to the medical profession Between 1 and 2% of all admissions to one Sanatorium in Missouri and 2% in Tennessee can be proved to have active pulmonary histoplasmosis by culture The symptoms of chronic pulmonary histoplasmosis resemble in every way those of chronic pulmonary tuberculosis namely cough sputum malaise mild fever and loss of weight Bloody sputum is infrequent In general the patient s symptoms are mild and are characterized by exacerbations and remissions During the remissions the patient feels relatively well The exacerbations are characterized by the onset of what is thought by the patient to be influenza at which time new areas of the lung are found to be involved The patients are usually in the older age group and many show a record of previous hospital admissions The disease is chronic and tends to progress over the years (Fig 8A and 8B) Recent studies of 87 cases followed for three years or more indicated that 79% had progressed involving more areas of the lung than were involved when first observed (Fig 9) The duration may be for as long as 20 years depending on the rapidity of progress of the disease Relapses are usual and many of these patients obviously would be found in sanatoria The laboratory tests show positive skin and serological tests and positive results by culture The sputum is not infrequently loaded with organisms and no difficulty is encountered in most cases in isolation



Fig 7B The calcification which appears with healing 4 years later makes evident the extent of the pulmonary involvement This boy was absent from school for an entire year with cough headache and painful joints Both tuberculosis and histoplasmosis were considered but no treatment instituted because of slow clearing of the lesions (Reprinted by permission from Rabin Coleman B *et al* *Roentgenology of the Chest* Springfield Ill Charles C Thomas Publisher 1958)

adults or adults in contrast to children This may be because of heavier exposure of the adults

Histoplasmoma A development of extreme interest in regard to the ultimate fate of the acute pulmonary infiltrations due to Histoplasma is the finding of pulmonary coin lesions or histoplasmoma as they have been called by Katz (22) The original connection of these granulomata to histoplasmosis was first reported by Puckett (23) who indicated that histoplasmoma is the most common granulomatous lesion occurring in the lung Experience in a number of different surgical clinics has shown that up to 80% of the coin lesions removed because of confusion with possible lung cancer have revealed Histoplasma capsulatum with proper staining Since calcification commonly develops in Histoplasma infection the presence of calcification is evidence in favor of the histoplasmic origin of these coin lesions



Fig 9 N M age 56 from an Illinois hospital Onset 15 months before with chest cold Histoplasmin skin test negative Complement fixation test for histoplasmosis positive Cultures positive for H capsulatum Bilateral pulmonary infiltration and cavitation involving the upper half of the right lung and medial half of left lung (By permission of the American Academy of General Practice)

Fig 10 W M age 69 from a Kansas hospital Tuberculin negative histoplasmin positive Four negative sputums three negative gastrics for tubercle bacilli X ray shows infiltration and cavitation right upper lung scarring and calcification left base Histoplasma capsulatum repeatedly isolated from the sputum (By permission from the American Academy of General Practice)

circulation that the patient dies of circulatory or respiratory difficulties more than from active histoplasmosis However in many patients the disease progresses and eventually disseminates just prior to death

ACUTE DISSEMINATED HISTOPLASMOSIS

In many ways it is unfortunate to separate acute disseminated histoplasmosis from acute pulmonary histoplasmosis since the *portal of entry in most cases is presumed to be the lungs* However Christie (25) has indicated that it is also possible that some cases are infected by way of the gastrointestinal tract At any rate the characteristics of disseminated disease are that the organism is dissemi

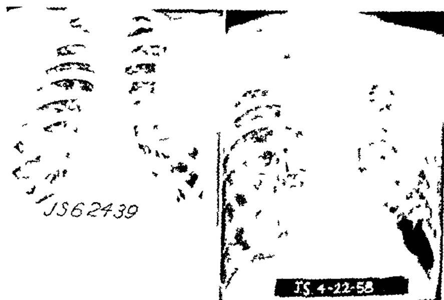


Fig 8A W M age 59 years native of Iowa X ray June 6 1939 shows marked confluent infiltration of the left midlung with a very large cavity medially and other possible cavities Scattered infiltrates are seen in the right lung especially the upper lobe Patient had had known chest disease for 3 years suspected of being tuberculosis but never proved

Fig 8B *X ray taken 19 years after Figure 8A* Marked progression of the disease on the left is seen with destruction of most of the left lung Apical infiltration and cavitation is seen on the right The patient has negative *tuberculin and positive histoplasmin skin tests His sputum has been repeat* edly positive for H capsulatum and his serological tests for histoplasmosis are positive Workup for tuberculosis negative

of the organism from the sputum The x rays in this type of disease show fibrotic infiltration usually of the apical areas of the lungs progressing to cavitation Bilateral pulmonary cavitation is ex tremely common indeed typical as the disease advances Calcified lesions are *frequently observed in the hilar areas suggesting that* perhaps this is a re infection type of disease since many of these patients are negative to tuberculin (Fig 10)

The course of the disease is chronic and usually terminates fatally although the progressive *involvement of the lungs may* result in so much destruction of lung tissue and obstruction of



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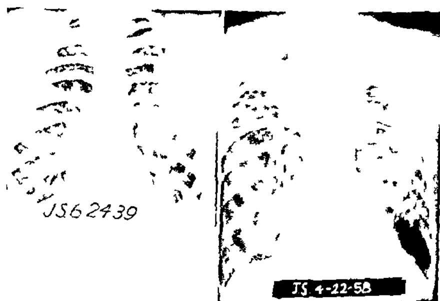


Fig 8A W M age 39 years native of Iowa X ray June 6 1939 shows marked confluent infiltration of the left midlung with a very large cavity medially and other possible cavities Scattered infiltrates are seen in the right lung especially the upper lobe Patient had had known chest disease for 3 years suspected of being tuberculosis but never proved

Fig 8B X ray taken 19 years after Figure 8A Marked progression of the disease on the left is seen with destruction of most of the left lung Apical infiltration and cavitation is seen on the right The patient has negative tuberculin and positive histoplasmin skin tests His sputum has been repeatedly positive for H capsulatum and his serological tests for histoplasmosis are positive Workup for tuberculosis negative

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CHRONIC DISSEMINATED HISTOPLASMOSIS

This type of disease is just becoming recognized and is characterized by the presence of disseminated disease of a rather chronic nature Indeed cases of this type have been known to live for three or four years with local or generalized evidence of Histoplasma infection Chronic dissemination appears to be more common in adults than in children This type of disease is most frequently found among patients suffering from other diseases such as tuberculosis Hodgkin s disease leukemia or similar chronic diseases The disease is extremely difficult to differentiate from the lymphomatous disorders because of the enlargement of the liver spleen and lymph nodes which may occur In addition the growth of the fungus in the bone marrow may distort the blood cell picture so as to resemble leukemia even further

In many cases of chronic disseminated histoplasmosis the adrenal glands may be invaded and indeed destroyed by the fungus This results in the addition of Addison s disease to the patient s symptoms Hence easy fatigueability low sodium levels in the blood and hyperpigmentation of the skin should be looked for and indeed expected In many cases the adrenals at autopsy appear completely destroyed by the growth of the fungus Quite frequently the disease is first diagnosed by the finding of a mucosal or laryngeal ulcer which when biopsied reveals H capsulatum In most of these cases when careful examination is done other signs of dissemination will be found such as enlargement of the liver and spleen positive serological tests positive blood cultures etc In our experience these lesions are not benign and while the disease may be present in a chronic form for periods of several years the patients all ultimately died of histoplasmosis with the exception of a few in the past year who have been treated Contrary to our experience Christie (3) reports that some mucocutaneous lesions are benign Patients with dissemination may or may not show pulmonary involvement They quite often have other diseases and their course is in general prolonged However as mentioned before the disease does tend to disseminate terminally and in our experience usually end fatally

We now come to discussion of the less frequent types of histo

nated throughout the body by way of the blood stream while the pulmonary manifestations are few or absent It has slowly emerged that cases of acute disseminated histoplasmosis are not always fatal as previously suggested (26) There appear to be 3 possible outcomes—the first—recovery the second—acute dissemination with rapid fatal outcome and the third—chronic dissemination with ultimate death but only after as much as 4 years The characteristics of the acute disseminated disease are that lung lesions are often relatively few or inconspicuous whereas the signs of systemic disease spiking temperature often up to as high as 105 or 106°F hepatomegaly and splenomegaly are common In the predominantly gastro intestinal type diarrhea is a common symptom In disseminated disease the chest x rays may be negative although usually at autopsy pulmonary foci are discovered Dissemination is more common among the very young and very old possibly because of lowered resistance in these age groups The frequency of this type of disease is unknown since misdiagnoses are not uncommon and often diagnosis is made only at autopsy The duration in acute disseminated histoplasmosis is ordinarily 6 weeks or less If the duration is more than 6 weeks the case is arbitrarily classed as chronic In disseminated disease skin tests are often negative possibly because of the high temperature or prostration Serological tests are almost always positive as is culture of the blood or bone marrow Prognosis based on the outcome which is of course merely a measure of the resistance of the host divides these cases into benign and progressive Even in the earliest reports of histoplasmosis cases are found of disseminated disease in which recovery has taken place (27) With the increasing recognition of the disease the frequency of non fatal dissemination is increasing (See chapter on epidemiology) However it still remains true that in the progressive type of acute disseminated histoplasmosis the prognosis is extremely grave and most of these cases die in a short time In children acute disseminated progressive disease is quite frequently manifest by diarrhea and predominantly gastro intestinal symptoms This occurs occasionally in adults but is unusual as the author has only seen two cases of this type in an experience involving several hundred cases

This has been reported in a number of cases and we have seen several cases ourselves (35) In addition if the nodes happen to surround the bronchus constriction is not uncommon with healing and atelectasis and bronchiectasis as the result Sometimes the bronchiectasis is merely mechanical and is not accompanied by active

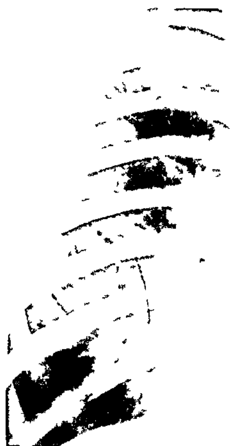


Fig 11 W M age 16 Chest x ray July 9 1958 reveals thick walled cavity with apparent fluid level in right midlung Several thin walled areas suspected of cavity nearer hilus Other areas in first interspace and hilus appear to be calcifying Tuberculin negative histoplasmin positive serologic tests for histoplasmosis positive This represents one in a series of x rays showing several areas of infiltration alternating with thin or thick walled cavities

plasmosis which perhaps should not be dignified by the terms of types These are varieties of disease whose etiology is unquestioned but frequency and importance are as yet unknown

Cutaneous Histoplasmosis This will be discussed as either primary or secondary Due to the studies of Wilson in coccidioido mycosis (28) and Schwarz and Baum (29) and Wilson (30) in blasto mycosis there has been considerable interest in primary or inoculation histoplasmosis This type is characterized by a localized chancre like lesion at the point of inoculation accompanied by enlargement of the regional lymph nodes It is extremely uncom mon in histoplasmosis Curtis and Cawley (31) having reported the only 2 known cases is penile lesions We have seen one other possible case in a laboratory washer who developed an indurated ulcer on his finger with regional lymphodenitis while handling Histoplasma in the laboratory His skin test converted to positive an x ray lesion developed in his lung and his serologic tests for histoplasmosis became positive Three other cases showing only cutaneous involvement but without the chancre form lesion or regional adenitis have been reported 2 from England (32) and one from Australia (33)

Secondary involvement of the skin and mucous membranes accompanying dissemination is not uncommon occurring in per haps a third of the reported cases Such lesions are usually part of a progressive disease and the outlook is poor although Loosli (4) states that transitory dissemination of the fungus with skin and mucous membrane lesions have been observed during the course of acute non fatal pulmonary histoplasmosis acquired in the labora tory Christie (3) also reports a non fatal non progressive form with mucocutaneous lesions This subject has been reviewed by Baum *et al* (34)

Broncholiths, Bronchiectasis and Middle Lobe Syndrome It would be expected that with the frequency of calcification so com mon in histoplasmosis (30% of positive skin test reactors) one might frequently find lesions in the hilar lymph nodes This may lead with the passage of time to the breakdown of these calcified lymph nodes which discharging into the bronchus produce broncholiths

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11 Furcolow M L. Gentheroth W C an l Willi M J The frequency of labora tory ınfectıcns with *Histoplasn a capsulat* ı their clinical and x ray charac ter tıcs *J Lal & Cl n Med* 40 18 18 1J
I Furcolow M L an l Boyzm A Development of compler ent fıxing antıbod es and chest lesıons among histop la mın co verters I resented before Ep demı ology Sect n f Am I ub Healtl Assoc Oct 31 19 0 St. Louis Mo
15 Furcolow M L and Ne l eter Ep ı len ol gıc a pects of hı t p lasmosis *Am J Hyg* 65 9C1 2 0 19
14 Sabın A P Miliary granul omatoı pneumonitıs ın a group of men e posed to pigeon excreta *T 4 th N t Tuberc 4 s p* 80 1J I
1 Wa hl urn A M Touhy J H an l Da ı E I Ca e kness A new disease entıty *Am J P b Healtl* 38 1 1 15 C 1948
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3 Puckett T F Iulmonary histoplasmosis A study of t enty one ca e w th ler tıficat on of *H ap l t ı* n esected le on *A Pev Tuberc* 6 453 4 6 19 4
4 Walls K F colow M L and Lehan P H Histoplasmosis as a problem ın tuberculos nato ums tl ro ghout the Un ted States *J L b & Clın Med* 51 C 0 19 8
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7 Bunnell I L and Fu olow M L A po t n t n p o d c es of hı toplas mos s *Pub H alth Pep* 63 11 31C 1948
8 Wılson J W Smith C E an l Plunkett O A Pr m ry cı taneous cocc dıo domycos The criteria for dia nos and a repo t cf a case *Californ Med* 9 933 39 19 3

histoplasmosis (36) At other times active histoplasmosis accompanies bronchiectasis with positive sputum and serologic tests The exact degree to which histoplasmosis is important as a cause of bronchiectasis has not been fully determined

Another pathological entity of extreme interest is mediastinal fibrosis leading to obstruction of the superior vena cava This entity has only recently been recognized It was first reported by Gillespie (37) Miller (38) has reported two cases and has 2 additional cases (39) and one has been seen in Cincinnati hospital while Lt Col Richard R Taylor at Fitzsimmons Hospital has 7 additional cases (40) This type of disease is characterized by progressive obstruction of the superior vena cava with or without active pulmonary disease In all of these cases satisfactory pathological evidence of histoplasmosis is demonstrable However the pathological picture is characterized by development of extensive firm fibrosis which is extremely difficult to handle surgically Whether this represents chronic infection or whether it is a residual type of disease is unknown but it does appear to be a problem which should be brought to the attention of practitioners Speaking of the development of fibrosis in this type of infection one cannot help but wonder about the importance of histoplasmosis in chronic pulmonary fibrosis

The shelling out of infiltrations in histoplasmosis has not been frequently reported However White *et al* (41) report the occurrence of 3 cases in which cavities developed in nodular lesions We have seen several cases of this occurrence and one is shown in Fig 11 However it is quite apparent from many reports that the shelling out of nodular lesions is rare in histoplasmosis in marked contrast to coccidioidomycosis

REFERENCES

1 Smith C E Beard R R Whiting E G and Rosenberger H G Varieties of coccidioidal infection in relation to epidemiology and control of the diseases *Am J Pub Health* 36 1394 1402 1946
2 Furcolow M L The clinical diagnosis of histoplasmosis *Postgrad Med* 0 349 364 1956
3 Christie A The disease spectrum of human histoplasmosis *Ann Int Med* 49 544 555 1958

THE DIAGNOSIS OF HISTOPLASMOSIS

W D Sutliff

The diagnosis of histoplasmosis has become practical and most internists in the area of high endemicity have recognized one or more cases The greatest strides in the past ten years have been made in the recognition of the clinical manifestations and representative series of various clinical types have been reported with sufficient detail to form the basis for diagnosis Although definitive etiologic diagnosis of histoplasmosis remains dependent upon specific methods diagnostic procedures such as cultures of Histoplasma capsulatum serologic tests and skin tests are no longer considered exceptional or difficult Only asymptomatic cases or cases with another obvious specific disease such as tuberculosis bronchiectasis sarcoid like or middle lobe syndrome due to H capsulatum are likely to escape recognition today

The change in our diagnostic capabilities with respect to histoplasmosis is of such recent origin that it may not be out of place to describe some of the steps taken in arriving at the present situation Histoplasmosis was first described as a rare malignant tropical infection Isolation of the microorganism was achieved 29 years after its first description During the first forty years few cases other than those terminating fatally were described The protean nature of the manifestations of fatal cases led to the recognition and report of one or a few cases at a time The various clinical types of histoplasmosis were recognized only after groups of cases were collected Acute disseminated histoplasmosis in its severe form was described first (1) Acute pulmonary histoplasmosis was sought and found after epidemiologic studies showed that such cases probably occurred in large numbers in the endemic areas (2 3) The symptoms and physical signs of acute pulmonary histoplasmosis were described as a result of surveys of normal populations (4) as a result of the observation of epidemics (5) and as a result of the description of occupational infections in laboratory workers (6) Chronic pul

29 Schwarz J and Baum G L Blastomycosis *Am J Clin Path* 21 999 1099 1951
30 Wilson J W Cawley E I Weidman F D and Gilmer W S Primary cutaneous North American blastomycosis *AMA Arch Dermat* 71 39 45 1955
31 Curtis A C and Cawley E P Genital histoplasmosis *J Urol* 57 1947
32 Symmers W St C Localized cutaneous histoplasmosis *Brit M J* 2 790 793 1956
33 Johnson D W and Derrick E H Histoplasmosis Report of an Australian case *M J Australia* pp 518 523 1948
34 Baum G L Schwarz J Bruins Slot W J and Straub M Mucocutaneous histoplasmosis *AMA Arch Dermat* 76 4 8 1957
35 Furcolow M L Unpublished data
36 See Figure 6
37 Gillespie J B Superior vena caval obstruction in childhood Report of a case secondary to histoplasmosis *J Pediat* 49 390 325 1956
38 Miller D B Allen S T Jr and Amidon E L Obstruction of the superior vena cava presumably due to histoplasmosis *Am Rev Tuberc & Pulm Dis* 77 818 857 1958
39 Miller D B Personal communication
40 Taylor Lt Col Richard R Personal communication
41 White F C Chronic pulmonary disease in histoplasmin reactors A review of 229 cases discovered through chest clinic examinations *Am Rev Tuberc* 72 274 295 1955

(3) Chronic pulmonary histoplasmosis (usually reinfection)
(4) Chronic extrapulmonary histoplasmosis (reinfection)
  a Mucocutaneous
  b Of endocardium
  c Of adrenal glands
  d Of central nervous system
  e Of other organs or tissues
(5) Disseminated histoplasmosis (primary or reinfection)

Asymptomatic histoplasmosis refers to cases in which evidence of infection often retrospective is present but in which illness due to histoplasmosis is not recognized Since 80% of adult populations in areas of high endemicity may be found to react positively to histoplasmin skin tests the number of such asymptomatic cases must be great but they are generally outside the practical interest of either patient or physician A person who has converted the histoplasmin skin test from negative to a definitely positive reaction can be assumed retrospectively to have experienced a sub-clinical infection with H capsulatum Likewise a retrospective diagnosis was made in the presence of striking calcific nodules (8) in persons living in an area of high incidence as follows (1) the primary complex with a comparatively large calcified nodule anywhere in the lung parenchyma and a calcified nodule in the adjacent hilar lymph node sometimes 1 to 4 cm in size with snail shell like convolutions (2) disseminated small rounded miliary calcified nodules evenly distributed in the lungs (3) similar miliary calcified nodules in the spleen or (4) comparatively large solitary nodules in the pulmonary parenchyma which were due to inactive histoplasmosis The retrospective diagnosis of previous asymptomatic infection can be more firmly established following an epidemic when the patient has had opportunity of exposure to a proven source of infection Data on symptomatic infections in the literature usually concern patients with pulmonary infections but it is assumed because of lesions sometimes found in the gastrointestinal tract in disseminated disease that some part of the asymptomatic infections may also depend upon the gastrointestinal tract as the portal of entry It should be noted while considering asymptomatic infections that the recognition of splenic calcifications as residual manifestations

monary histoplasmosis had to be distinguished from chronic pulmonary tuberculosis both by exclusion of tuberculosis and by definitive diagnosis of histoplasmosis following surgical excision of lesions and the demonstration of H capsulatum in pulmonary tissue (7) A slow accumulation of individual case reports defined the clinical manifestations of chronic disseminated histoplasmosis and extrapulmonary forms such as the mucocutaneous variety

NOSOLOGY

As a result of the separate description of the clinical varieties of histoplasmosis their nosology has not been uniform Physicians describing the disseminated disease used the concept of a spectrum of clinical manifestations for which two extremes were clearly defined such as mild localized disease at one end of the spectrum and severe disseminated disease at the other Another terminology emphasized mild and severe forms of disease and the presence or absence of progression Another terminology defined primary and reinfection histoplasmosis as these terms are used for tuberculosis The author has tended to apply the principle that anatomic location of the lesions together with the acute or chronic character of the course take precedence over other designations For the purpose of this chapter such a plan has functional advantages in that the first step in diagnosis is the recognition of a group of symptoms and physical signs relating to the anatomic site of involvement and it is on this basis that etiologic studies are then carried out Accordingly syndromes are emphasized in the plan that is outlined below The primary or reinfection character of the case is indicated only by additional adjectives as it is determined following the accumulation of complete evidence Prognostic concepts such as progression are left for separate consideration in each case as they are related more directly to the presence of dissemination or to serious complications The terminology below is used for the purpose of making diagnosis easier in this chapter and without prejudice to the many other excellent schemes for classifying the clinical types of histoplasmosis as follows

(1) Asymptomatic histoplasmosis (primary)
(2) Acute pulmonary histoplasmosis (usually primary)

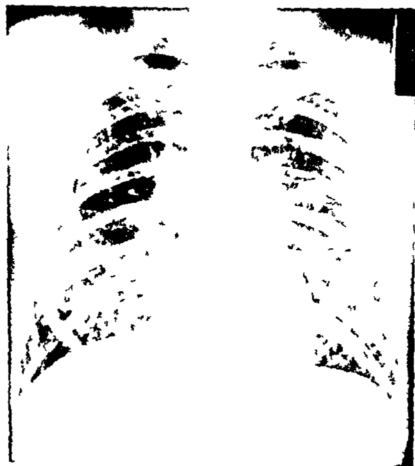


Fig 1 Roentgenogram of chest Acute pulmonary histoplasmosis seventh day Diffuse mottled infiltrations and moderate hilar lymphadenopathy (From Kier John H et al J A M A 155 1230 1239 1954 Reprinted by permission of author and journal)

was no history of previous histoplasmosis and no residual pulmonary or lymphatic calcified nodules and specific skin and serologic tests were negative during the first three to four weeks of illness In a few acute pulmonary cases the evidence indicated reinfection There was evidence of previous histoplasmosis consisting of a primary complex or other calcific lesions and specific skin and sero

of primary infection usually with concomitant pulmonary residual lesions is evidence that generalization occurs in some asymptomatic infections

Acute pulmonary histoplasmosis is recognized by symptoms and signs that resemble the course of atypical pneumonia The history of exposure to infected materials from which H capsulatum has been cultured is sometimes available and aids in the diagnosis The following sites and activities (9) of patients have been impli cated at the point of source of epidemics and may be considered of possible diagnostic significance in the histories of patients shovel ing pigeon excreta at a schoolhouse or in a water tower gathering chicken manure cleaning or tearing down a chicken house work ing about turkey roosts cleaning farm buildings making a wood fire in a storm cellar or in a farmhouse children playing about a farm or in a hollow tree digging in soil in the woods to get fish worms or in a cave sifting garden dirt It has been postulated following contemplation of this data about the point of source of epidemics that the presence of dusty material in a site that has been alternately damp and dry and is enclosed are features common to many such sources

Patients with acute pulmonary histoplasmosis became ill with fever sweats nonproductive cough and fatigue from 7 to 21 days after exposure Some of the patients had chills at the onset burning chest pain and anorexia some lost weight and were very weak The symptoms disappeared in from a few days to two weeks in most cases Roentgenographic pulmonary lesions were often diffuse mottled densities from 1 to 2 cm in diameter uniformly distributed throughout both lung fields Some had a single or a few infiltra tions located in any part of the lung field All had enlarged hilar lymph nodes either bilateral or unilateral The pulmonary changes tended to persist for from one to three months or in some cases residual granular thickening of the pulmonary markings was noted The sites of the lesions tended to calcify after 18 months to three years or longer both in the lung parenchyma and in the lymph nodes

The majority of acute pulmonary cases of histoplasmosis de scribed in the literature appeared to be primary infections There

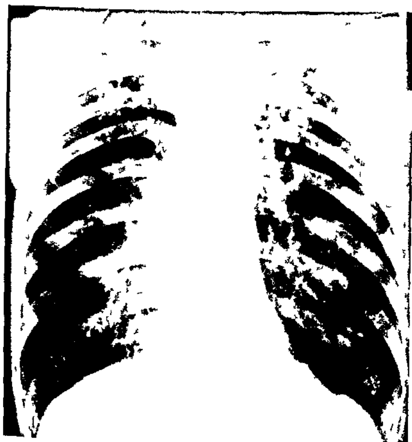


Fig 9 Roentgenogram of chest Chronic pulmonary histoplasmosis 19 months after onset Bilateral apical fibrocavitary disease Cavity 4 cm in diameter on right Multiple cavities 1.5 cm in diameter on the left Miliary calcified nodules on the right and angular calcified nodules 0.5 cm in diameter in hilum and in lesion on the left Left hilum displaced upwards

remarkable in that little extrapulmonary involvement was found in cases of chronic pulmonary histoplasmosis H capsulatum was present in large numbers and was readily isolated from the sputum of cases with chronic fibrocavitary histoplasmosis Serologic tests and histoplasmin skin tests were positive with very few exceptions

Confirmation of the diagnosis of chronic pulmonary histoplasmosis was unsatisfactory in from one half to two thirds of the cases

logic tests were positive when first performed In such cases the pulmonary lesions were apt to be small and sharply demarcated sometimes miliary in appearance and without hilar lymphadenopathy

Acute pulmonary histoplasmosis cases both primary and reinfection had a good prognosis

Collection and culture of material to which the patient was exposed was often of value in identifying H capsulatum as the etiologic agent (see under exposure below) Sputum gastric washings bone marrow or biopsied lymph nodes especially if obtained early in the course of the symptomatic disease were only occasionally the source of confirmatory positive cultures

Chronic pulmonary histoplasmosis was recognized more frequently in hospitalized adults than other clinical forms of histoplasmosis The following thumbnail description of chronic pulmonary histoplasmosis was based upon a series of cases with fibrocavitary lesions diagnosed with the aid of positive cultures of H capsulatum (10) The disease occurred most often among farmers It began insidiously or with symptoms of pharyngitis or pneumonia usually with fever The course was chronic with chronic cough mucopurulent sputum weight loss chest pain weakness and fatigue as the principal symptoms Roentgenology showed the lesions were nearly always in the apical portion of one or both lung fields Marked fibrosis of the pulmonary lesions led to displacement of the trachea and hilar structures toward the areas of maximal involvement Cavities were demonstrable often multiple and small in size sometimes large and thin walled resembling bullae Acute or chronic complicating pulmonary infections associated with the presence of bacteria such as Klebsiella pneumoniae or Micrococcus pyogenes aureus were observed as well as pulmonary tuberculosis The most frequent complications were emphysema and fibrosis associated with dyspnea on exertion The course tended to be stable in over half the cases An unfavorable outcome was usually associated with complications such as infected pulmonary bullae emphysema etc but not with active spread of histoplasmosis Likewise repeated observations such as bone marrow and lymph node cultures and postmortem studies were



Fig 3 Chronic disseminated histoplasmosis Mucocutaneous papulo-ulcerative lesions of lips Liver spleen bone marrow and adrenal glands also involved

In mucocutaneous histoplasmosis (11) the principal sites of involvement were the oral cavity the lips the faucial pillars the tonsils the pharynx the tongue the buccal mucosa the larynx and the skin where ulcers or nodules were seen The lesions tended not to be tender but developed as papules and ulcerated leaving a firm margin without cellulitis or secondary bacterial infection In some cases marked involvement of the regional lymph nodes simulated lymphadenitis due to tuberculosis Hodgkin s disease lymphoma or sarcoid

In endocardial histoplasmosis (12) the signs of changing murmurs high intermittent fever emboli hematuria clubbing of the fingers anemia or splenomegaly dominated the picture as described in 7 of 10 cases by Merchant Valves involved in 10 cases were aortic 5 tricuspid 3 mitral 1 and mitral and aortic 1

Histoplasmosis of the adrenal gland (13) reproduced exactly the symptoms of Addison s disease due to the complete destruction or replacement of the gland by a caseous mass Such patients were apt to have some lesions of mucocutaneous histoplasmosis but it

because sputums and other materials submitted for cultures were persistently negative and bronchoscopic specimens and bronchoscopic observations and bronchograms disclosed no confirmatory information and no reasonable diagnostic alternative In such cases it was necessary to consider the diagnosis less well established than in cases with positive cultures The term presumptive was added to the diagnosis of chronic pulmonary histoplasmosis when examination was thorough and was negative for other conditions

Other chronic pulmonary lesions were less common and generated different sets of symptoms and x ray findings Pleural effusions due to H capsulatum were rare The histoplasmoma a coin lesion usually with stippled or central nodular or concentric rings of calcific material occurring in any part of the lung was sometimes asymptomatic but was often studied thoroughly to rule out neoplasm Histoplasmosis of the mediastinal lymph nodes with mediastinitis was sometimes sufficiently extensive to produce obstruction of the superior vena cava Histoplasmosis of the lymph nodes adjacent to the major bronchi also tended to produce obstruction which was reported more commonly to affect the right middle lobe with the production of middle lobe syndrome and in some cases bronchiectasis Specimens of excised pulmonary lesions or infected lymph nodes were usually necessary for the definitive diagnosis through the demonstration of H capsulatum in these forms of chronic pulmonary histoplasmosis

Nearly all patients with chronic pulmonary histoplasmosis lived and worked under conditions of repeated exposure to possible sites of infection usually about farms Most did not recall any one heavy exposure before the onset and in some cases the onset was so gradual that it could not be precisely dated It seems reasonable to suppose that endogenous reinfection was often the cause of chronic pulmonary histoplasmosis However no definite conclusion as to the occurrence of endogenous or exogenous reinfection was usually possible in individual cases

Extrapulmonary histoplasmosis was occasionally localized in one or a few organs or sites namely mucous membranes or skin endocardium adrenal gland meninges etc The symptoms were those related to the site of the lesion

1945 Following 1945 a larger proportion of the reported cases of all types were reported annually as recovering from histoplasmosis Such recoveries were more frequent in adults and were apt to be associated with less acute and less extensive dissemination The prognosis was related to the acuteness of the symptoms and to the degree of dissemination

When the gastrointestinal tract (16) was involved the jejunum cecum and colon were involved in disseminated histoplasmosis and diarrhea melena anorexia nausea and vomiting sometimes dominated the clinical picture Shull (16) described involvement of the small bowel large bowel or liver in 16 out of 21 cases coming to postmortem in Nashville Tennessee Ulcers with a firm base and margin were present in or about the anus or were visible on proctoscopy In some cases broad flat papules were described in normal bowel mucosa in some of which ulcers developed One case of fatal hemorrhage from a duodenal ulcer with active lesions of histoplasmosis was described occurring during the course of disseminated histoplasmosis

Bone marrow specimens were the most profitable source for demonstration of H capsulatum in disseminated histoplasmosis Microscopic examination and culture of lesions were also productive such as lymph nodes liver spleen lung mucocutaneous lesions The stools sputum or blood were also positive in some cases

AGE, SEX RACE

Cases of histoplasmosis occurred at all ages There was a relatively high incidence of the disseminated form of the disease in the first year of life which equalled that in any other decade Incidence was again high in the fifth and sixth decades Up to the age of ten there was no sex difference but after puberty males predominated Conversion of skin reaction to positive occurred most frequently from age four through age eighteen in endemic areas No racial predisposition was reported

appears possible that some of the cases of adrenocortical insufficiency without apparent complicating lesions of either tuberculosis or histoplasmosis may have had histoplasmosis and the relative proportion has not been determined It was recommended that cases of Addison s disease should be examined both for tuberculosis and histoplasmosis

Central nervous system infection (14) was reported as dominating the clinical picture in a case of histoplasmosis

The occurrence of constrictive pericarditis (15) in patients with no evidence of tuberculosis but with positive skin tests for histoplasmosis led to the discussion of the possibility that histoplasmosis was the initial cause of chronic constrictive pericarditis in some of the patients in whom evidence for other etiology was lacking

Specimens obtained by biopsy from lesions or blood specimens in the case of endocarditis were necessary for definitive diagnosis through demonstration of H capsulatum

Disseminated histoplasmosis varies from a mild to a severe acute or chronic disease The acute generalized forms occurred mostly in children and involved the liver and spleen and bone marrow as well as other organs and tissues The general symptoms as described by Parsons and Zarafonetis in 1945 were low and irregular or moderate fever present in 95% of the cases loss of weight in 40% with emaciation in 30% moderate to severe anemia of the hypochromic type in about 90% palpable liver and spleen in 75 to 80% lymphadenopathy in 70% leukopenia in 60% albuminuria in 60% and lesions in the lungs in 60% Roentgenology of the lungs was negative during life in two thirds of the disseminated cases Dense massive involvement or diffuse mottled densities predominated Symptoms referable to the gastrointestinal tract occurred in 25% ulcerative lesions of the oropharynx in 30% of the skin in 15% and lesions of the larynx in 15% The endocardium the adrenals and central nervous system were less often involved The disease when generalized had a duration of from a few weeks to six to eight months the course was usually shorter in young children As stated above the fatality rate in disseminated histoplasmosis was about 100% in cases reported from 1905 through

growth It grew well at room temperature on blood agar plates prepared with the addition of penicillin and streptomycin to control bacterial contamination Collection of material such as soil from the environment of patients in which the microorganism was present in the form of spores and mycelial growth did not require fresh specimens but the culture required special methods in which mouse inoculation provided the most favorable medium Microscopic examination was best carried out on exudates with the aid of Giemsa Wright or Wilson staining methods H capsulatum in biologic material appeared as a yeast from 2 to 5 microns in diameter with scattered irregular masses of nuclear protoplasm The yeast cells were packed in clumps within phagocytic cells but were also free in the purulent exudate Growth in the incubator was yeast like without the production of mycelium or spores At room temperature the growth was mycelial with the production of typical tuberculate chlamydospores The demonstration of these characteristic structures was the object of all culture methods since they were diagnostic of the organism

Biopsies made for the differentiation of granulomatous and neoplastic disease were often the source of definitive evidence of histoplasmosis Surgeons and pathologists learned to routinely split biopsy specimens into equal sized and equally representative specimens and carry out full etiologic studies by culture and appropriate histologic technique applied to separate parts of the tissue Although the microorganisms were sometimes readily observed with hematoxylin eosin stains special periodic acid stains or silver impregnation methods were more apt to disclose the presence of H capsulatum in tissue section Cultures were sometimes positive in tissues in which the microorganism was not demonstrated microscopically and conversely microorganisms resembling H capsulatum were sometimes observed that were not viable in culture Both histologic and culture methods were therefore needed for the maximum number of positive results Under some circumstances the appearance of H capsulatum was atypical in that large forms appeared similar to tissue forms of Blastomyces dermatitidis Coccidioides immitus or Cryptococcus neoformans At other times the small forms of these three systemic mycoses resembled the usual

EXPOSURE

History of exposure in a characteristic site or occupation was sometimes of value in diagnosis of acute pulmonary or acute disseminated histoplasmosis History of residence in an area of high endemicity was also sometimes of value Skin test surveys and tabulation of the location of cases and epidemics of cases of histoplasmosis delineated clearly a number of areas of relatively high incidence (17) In North America the whole Mississippi Valley was one of these as was the St Lawrence River Valley and a portion of the State of Virginia in the Southeast and North Carolina in the Northwest Other areas of high incidence were most parts of Latin America and Southeast Asia and Indonesia A case of histoplasmosis seen in areas of lower prevalence was frequently associated with previous residence in one of these areas of high incidence There were small areas outside these locations however apparently scattered throughout the world where the infection could be contracted A history of residence in an area of high endemicity suggested the diagnosis of histoplasmosis but the absence of such history would not rule out histoplasmosis

DEMONSTRATION OF H CAPSULATUM

The demonstration of the microorganism was the most valuable single evidence of the presence of histoplasmosis No circumstances in which H capsulatum existed as an incidental contaminant in man have been described and it is reasonable to consider it to be a pathogen whenever it is recovered from human secretions or tissues Secretions exudates body fluids and tissues for examination for H capsulatum should be fresh if possible and in ample amount If necessary the antibiotics penicillin streptomycin cycloheximide should be added to suppress excessive growth of bacteria or saprophytic fungi The rapid disappearance of H capsulatum from sputum specimens described by Kurung may be minimized by refrigeration Tissues for culture should be thoroughly ground in a Ten Broeck homogenizer or other similar instrument The microorganism was not fastidious but occasionally required inoculation on a series of plates for initiation of

SEROLOGIC REACTIONS

The general association of positive serologic reactions with the course of active infection with H capsulatum was clearly demonstrated (18) Serologic tests such as the complement fixation with yeast phase antigen became positive in the third week of acute primary histoplasmosis and remained positive during the whole period of activity In a few cases these tests remained positive for years although clinical activity was slight or absent The tests were apt to become negative in the terminal stage of acute disseminated infections or in chronic well localized disease The value of the serologic test as a screening procedure was studied From 3 to 7% of cases (19) hospitalized in pulmonary disease hospitals were found to have positive serologic tests using yeast phase antigen and subsequent confirmation was obtained by culture in about 1% The antigens were not standardized nor manufactured for general use and serologic testing was thus limited by the availability of suitable antigens The tests when available offered a means for tentatively confirming the presence of histoplasmosis in cases where other methods were not available They were found most valuable in the diagnosis of some cases of acute pulmonary histoplasmosis or in presumptive chronic pulmonary histoplasmosis where positive cultures were not obtained

The differential diagnosis of histoplasmosis was dependent upon the isolation of H capsulatum or its recognition by microscopy Other specific methods such as skin tests and serologic reaction were sometimes valuable The following list of medical conditions were simulated by acute pulmonary histoplasmosis primary atypical pneumonia psittacosis Q fever influenza and bacterial pneumonia Chronic pulmonary histoplasmosis simulated other systemic fungus diseases benign or malignant primary or metastatic tumors chronic lung abscess bacterial pneumonia especially Klebsiella pneumonia bronchiectasis and hamartoma Chronic extrapulmonary histoplasmosis simulated carcinoma of the oral cavity bacterial endocarditis and other conditions corresponding to other sites of localization Disseminated histoplasmosis simulated other systemic causes of fever such as bacteremia brucellosis and neoplastic disease

H capsulatum microorganisms Since both the pathologic lesions and the microorganisms resembled one another cultures were always necessary to confirm the specific strain of fungus Cultures with the resulting identification of characteristic tuberculate chlamydospores when successful was considered the most specific method for the identification of H capsulatum

Skin tests using histoplasmin which had been standardized by comparison with a sample of standard potency were most valuable in areas of high incidence of histoplasmosis when they were negative and therefore tended to exclude the diagnosis of histoplasmosis In areas of low incidence the positive skin test was of more affirmative value The test had to be carried out with due attention to the avoidance of technical pitfalls and when thus performed the interpretation of the positive test was self evident Syringes and needles for histoplasmin skin tests should not be used for other skin test material such as PPD It was necessary to insure solution of the dried histoplasmin by inverting the vial to avoid any mixing with alcohol used for antisepsis to make a perfect intracutaneous injection rather than subcutaneous and to avoid or at least observe and repeat injections from which the fluid leaked Since most technical errors led to false negative tests careful repetition of unexpected negative tests was often indicated Reading of tests in millimeters of diameter of the induration at the 48th hour was recommended Within the first two to three weeks in primary histoplasmosis the skin test was negative and terminally in the disseminated disease no reaction was elicited The test was reported negative in a moderate number of chronic pulmonary or disseminated cases Under most circumstances however a positive skin test reaction to histoplasmin was present in patients with an infection with H capsulatum either active or inactive It was noted that there was a tendency for cross reaction of histoplasmin positive patients to B dermatitidis antigens and occasionally to coccidioidin but not as far as known with cases in which reactions to the purified protein derivative of the tubercle bacillus were positive The dilutions of fungal antigens used for skin tests were chosen so that they manifested the least possible amount of cross reactions and the larger of any two conflicting tests were the more significant

nodules and showed discrete epithelioid tubercles Schaumann bodies and asteroid bodies The distribution of the lesions in lymph nodes spleen liver and lungs was similar Other examinations gave similar results negative PPD skin tests and reversed albumin globulin ratio In some cases the observers concluded that the patient had sarcoidosis with a secondary infection by H capsulatum In the majority of cases in which H capsulatum was demonstrated however the patients ran the course of disseminated histoplasmosis and the extent and character of the lesions indicated that the whole picture was based on H capsulatum infection Israel (20) in his exhaustive study of the subject concluded that few cases of histoplasmosis simulated sarcoidosis and that histoplasmosis like tuberculosis needed to be considered as one of the forms of granulomatous disease to be excluded before a definite diagnosis of sarcoidosis was made He recommended the use of skin tests biopsy with histologic examination and cultures and serologic tests for histoplasmosis in all cases of sarcoidosis

Disseminated histoplasmosis sometimes occurred as a terminal complication in the presence of lymphomas (21) The lymphadenopathy and other lesions such as hepatosplenomegaly and ulcers particularly in the oral mucosa made the recognition of a complicating disseminated histoplasmosis difficult Other fungus infections some the result of the invasion of fungi that were usually nonpathogenic were also found in patients with lymphomas suggesting that lymphomas under treatment with potent modern remedies including antibiotics steroids and anti neoplastic therapeutic agents were more susceptible to the dissemination of fungus infections Cultures and microscopic examination of the lesions and the tissues removed in the case of lymphoma should always be made with the possibility of fungus infection in mind

In summary histoplasmosis was most frequently diagnosed following the recognition of certain clinical syndromes as follows acute pulmonary histoplasmosis was suggested by atypical pneumonia especially if the x ray showed diffuse mottled infiltrations and hilar lymphadenopathy in patients with exposure to a rural environment or a dusty enclosed place Retrospective diagnosis of acute pulmonary histoplasmosis was made by observation of multi

Three conditions namely tuberculosis sarcoidosis and lymphoma were simulated by or complicated by histoplasmosis and were the subject of special studies

Chronic pulmonary histoplasmosis resembled chronic pulmonary tuberculosis but certain manifestations were more prominent in histoplasmosis than in tuberculosis Paraphrasing a popular song Anything tuberculosis can do histoplasmosis can do better Histoplasmosis was more frequent than tuberculosis in the areas of high endemicity Calcification fibrosis necrosis and cavitation with resulting distortion of the pulmonary architecture were more pronounced in histoplasmosis Chronic pulmonary histoplasmosis was less apt to progress than tuberculosis Histoplasmosis cases were often seen to develop a stable state of ill health persisting for years without local or general spread of the infection and to suffer more from pulmonary insufficiency than from active disease Since the differences were only differences of degree they were seldom of diagnostic value although they often served to raise the question of the presence of histoplasmosis The miliary pulmonary lesions of acute pulmonary histoplasmosis differed from miliary tuberculosis in their benign course and lack of response to antituberculous therapy Disseminated histoplasmosis was similar to hematogenous tuberculosis Because of the much greater incidence of tuberculosis it was necessary to consider the possibility of tuberculosis first If tuberculosis could be ruled out by repeated negative cultures of suitable sputum samples or negative Mantoux tests in patients living in an endemic area the presence of histoplasmosis was considered probable Histoplasmosis could be suspected however whenever clinical manifestations resembled those described above when tuberculosis could not be definitely confirmed by positive cultures when calcification fibrosis necrosis or cavitation were striking and when the response to antituberculous therapy was incomplete In some cases the diseases coexisted and both required specific confirmation by culture

It was not unusual for diagnostic problems to arise in the differentiation of disseminated histoplasmosis and sarcoidosis The granulomatous lesions of histoplasmosis when they were devoid of necrotic caseous degeneration duplicated the appearance of sarcoid

6 Furcolow M L Cuntheroth W R and Willis M J The frequency of laboratory infections with histoplasma cap ulatum their clinical and x ray character i tic J Lal & Cli Med 40 18? 18 19 ?

7 Sutliff W D Hughes F Ulri h F and Burkett I L Active chronic pulmonary hi topla m is 4 cl I t Me l 9 571 596 19 3

8 Br nsn S M an l Sch arz J Roentgen graphic patterns in histopla mosis 4 i Rev Tu l rc & Pulm Dis 6 143 131 19 7

9 Crayston J T an l Furcolow M L The occurrence of histoplasmosis in epidemics ep id m logic stud es Am J Pul Health 43 66 6 6 19 3

10 Sutl ff W D Experience with the course an l chem th erapy of chronic pul n ary h stopla osis 4 n Rev Tuberc & Pulm Dis 75 319 9 0 19 7

11 Baun C L Schwarz J Sl t J B an l Straul M Mucocutaneous histoplasmosis Arch Der mat 6 4 8 19

1 Merchant R K Louria D B Ceisler P H Edgcomb J H and Utz J P Fungal en locar litis review of the literature a l report f three ca es 4 n I t Med 48 1 ?6 13 8

13 Cr pell K R Larso W Haml J an l Hollifiel l G Ad li o s d sease associated w th histoplasmo s report of four ca es an l review of the literature Am J Med 0 ?3 ?9 19 6

14 Schulz D M H topla mosis of the central nervous system J A M A 151 519 1 19 3

1 McNerney J J Histopla mn eri it ity associated with pericar lial calcif cat on Am He rt J 5 609 611 19 6

16 Shull H J Human histopl smosi a l sease with pr tean manife tations often w th d gesti e systen nvol e nent Castroenterol gy 5 ?? 9 19 3

1 Edwar ls L Q an l Klaer J H W rld ide g ograpl ic d tribution of histoplas osis and l st pl m s sensitivity A J Tr J Med & Hygiene 5 ?3 ? 7 19 6

18 Crayston J T A stu ly of the c mplement fixat n reacti n in h toplasmos s J Lab & Cl M l 40 0 101 19

19 Sutliff W D a l Campbell C C Se ologic creen te ts fo the sy tem c mycose T 14th Co f nce on the Chem therapy of Tuberculosis pp ?85 88 19

0 Israel H L D Lam te E S nes M Will s W L and Ma melstein Capt A Ch on c l se nat l h stopl mo is an investigation of its relationship to s rcoidos A J M l I 2 ?60 19 2

1 Nelson N A Good nan H L and O ter H L The as ciation of h toplamo is nd lymphoma Am J M S 33 56 65 19 7

ple small calcified pulmonary nodules or single large pulmonary or hilar lymph node calcifications or splenic calcifications in patients with positive skin tests to histoplasmin Chronic pulmonary histoplasmosis was suggested by apical fibrocavitary lesions and a chronic stable course in an adult male over forty usually without evidence of active tuberculosis and usually readily confirmed by the presence of H capsulatum in the sputum Single nodular (coin) lesions removed surgically were also often due to H capsulatum infection Chronic extrapulmonary histoplasmosis was manifested by mucocutaneous granulomatous papules or ulcers or other localized lesions in the endocardium central nervous system adrenals etc Disseminated histoplasmosis was suggested by fever hepatosplenomegaly lymphadenopathy and leukopenia in infants and children and by multiple lesions mucocutaneous nodules or ulcers hepatosplenomegaly lymphadenopathy enterocolitis meningitis or signs of adrenocortical insufficiency in adult males Because of a similarity of histoplasmosis to tuberculosis in its various forms especially the chronic pulmonary disease tuberculosis is the first tentative diagnosis in many cases and histoplasmosis is recognized only after specific evidence for tuberculosis is found to be absent or equivocal Definitive diagnosis of histoplasmosis is based upon demonstration of H capsulatum usually from the environment in acute pulmonary histoplasmosis from the sputum in chronic pulmonary histoplasmosis and from the bone marrow or histoplasmosis lesions in disseminated histoplasmosis

REFERENCES

1 Parsons R J and Zarafonetis C D J Histoplasmosis in man *Arch Int Med* 75 1 1945

2 Christie A Histoplasmosis and pulmonary calcification *Ann New York Acad Sc* 50 1283 1298 1950

3 Furcolow M L Tuberculin negative histoplasmin positive disseminated pulmonary calcification *Postgrad M J* 8 15 20 1950

4 Furcolow M L Further observations on histoplasmosis mycology and bacteriology *Pub Health Rep* 65 965 19 0

5 Loosli C G Procknow J J Tanzi F Grayston J T and Combs L W Pulmonary histoplasmosis in a farm family a three year follow up *J Lab & Clin Med* 43 669-695 1954

from tuberculosis should also be considered. Demonstration of the etiologic agent, usually by culture or by animal inoculation, is the only criterion available for a definite diagnosis. Properly evaluated immunologic tests, however, often establish the diagnosis with a reasonable degree of certainty. The diagnosis of histoplasmosis in its various forms is being made with increasing frequency but it is probable that the great majority of cases, even when clinical symptoms are present, still escape detection. The number of cases diagnosed in any particular area is directly proportional to the degree of interest in the disease and often reflects the enthusiasm of some particular individual or group of investigators.

Finally, it may be said that the differential diagnosis of histoplasmosis is complex, multifaceted, somewhat controversial, difficult and in some instances impossible. Its importance to the patient, however, from the standpoint of medical and surgical treatment, fully justifies the vigorous attempts which are being made in many medical centers to achieve greater accuracy.

PULMONARY HISTOPLASMOSIS

The differential diagnosis of pulmonary histoplasmosis varies considerably with the type of infection and with the degree of severity. Table 1, reproduced from a recent publication by Baum and Schwarz (2), suggests a way in which some of the various clinical manifestations of the disease may be interrelated pathogenetically.

Asymptomatic disseminated pulmonary calcification in the mid western and south central United States is, in the majority of instances, associated with a positive histoplasmin test and negative tuberculin test (5). Occasionally single large calcified nodules in the lung with calcified tracheobronchial lymph nodes likewise appear on the basis of immunologic tests to be of histoplasmic origin (6). Healed histoplasmosis with calcification must therefore be differentiated from healed tuberculosis, as well as from the calcified lesions of healed coccidiomycosis and other fungal infections. This can be done only by the use of immunologic tests together with other available data.

The milder cases of primary pulmonary histoplasmosis tend to

DIFFERENTIAL DIAGNOSIS

HENRY PINKERTON

INTRODUCTION

Previous to 1945 histoplasmosis was rarely diagnosed during life and our concepts of the disease were based largely on clinicopathological studies of fatal cases These studies showed that its clinical manifestations were remarkably protean and could mimic primary disease of many different organs

With the advent of immunologic and cultural diagnostic methods it has become clear that a fatal outcome is relatively uncommon while asymptomatic infection may occur in 90% of those living in certain geographical areas (1) Non fatal infections of all degrees of severity are being recognized with increasing frequency (2) This broadening of the clinical spectrum of the disease has further increased the number of conditions which must be considered in its differential diagnosis

In 1945 Parsons and Zarafonetis (3) analyzed 56 cases of histoplasmosis in 40 of which there was generalized involvement and found reports of pulmonary involvement in only 34 instances In non disseminated cases however lesions are confined largely to the lungs In general the pathogenetic mechanisms methods of spread clinical vagaries time tables and pathologic sequences of histoplasmosis are rather strikingly similar to those previously established for tuberculosis and later for coccidiomycosis This parallelism extends even to such basic concepts as primary and secondary infection (4) the inter relationship of allergy and immunity and other complex and incompletely understood aspects of the host parasite relationship in chronic recrudescent infections

In the differential diagnosis of histoplasmosis tuberculosis must be given primary consideration since identical clinical and radiological pictures may be found in both conditions From this it follows that most of those diseases which must be differentiated

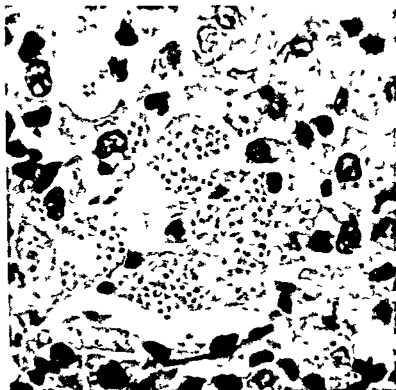
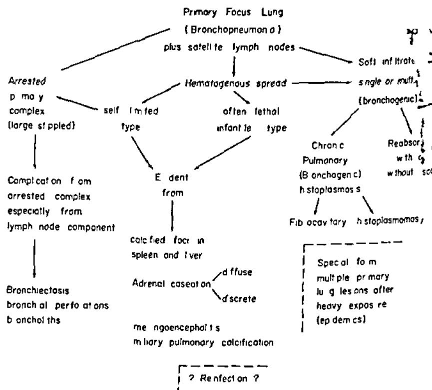


Fig. 1. Histoplasma capsulatum in adrenal epithelial cell adjacent to an area of caseation necrosis. Note the unstained capsule and the presence of signet ring forms. In a few organisms the central portion is uniformly stained.

test leads to a strong presumptive diagnosis of tuberculosis. Conversely, a negative tuberculin test with a positive histoplasmin test suggests histoplasmosis, particularly if geographic and other environmental factors harmonize with this diagnosis. Conversion of either test from negative to positive with the occurrence of the pulmonary infiltration greatly strengthens the diagnosis. When both tests are positive, the patient should be studied by all available means; occasionally a favorable response to chemotherapeutic agents routinely employed for tuberculosis may be the only specific evidence for making the latter diagnosis. The decreasing percentage of tuberculin reactors among the general population in recent

TABLE I



(Reproduced from New England Journal of Medicine by permission)

occur in epidemic form Radiologic findings may be negative and these cases resemble influenza and other respiratory infections (7) Since their recognition depends on cultural or immunologic tests particularly the complement fixation test (when performed at the proper time and in proper dilution) it is probable that the majority of these cases remain undiagnosed In the somewhat more severe cases positive roentgenologic findings include single or multiple areas of soft infiltrations enlarged tracheobronchial lymph nodes patchy areas resembling pneumonic infiltration and diffuse radiating infiltrations resembling those of sarcoidosis

In cases with areas of soft infiltration shown radiologically and with negative sputum examinations immunologic tests are of great importance A positive tuberculin test with a negative histoplasmin

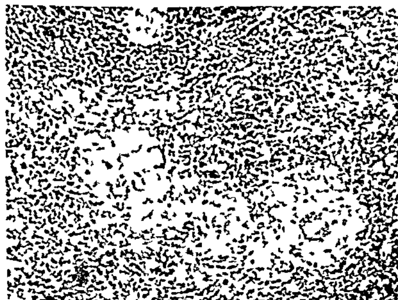


Fig 3 Non-caseous tubercle like lesions with central giant cells in spleen No organisms were found in these lesions

least in the mid western United States 3 to 7% of the patients have histoplasmosis without demonstrable acid fast infection In addition to these some patients have a mixed infection with the tubercle bacillus and the fungus The cases of pure histoplasmosis are discovered by studying carefully those patients who in spite of a completely typical clinical and radiologic picture have never had sputums positive for M tuberculosis The importance of these data is readily appreciated when one considers that antibiotic agents which are effective against M tuberculosis are ineffective and potentially harmful in mycotic infections

Chronic progressive cavitary histoplasmosis cannot be differentiated from tuberculosis by clinical or radiologic findings (2 8) diagnosis must depend on positive histoplasmin or other immunologic tests and the demonstration (by culture and animal inoculation) of the etiologic agent in the sputum (or other gastric washings) In febrile cases which eventually recover the organisms may be found temporarily in the blood or bone marrow (9) Often the

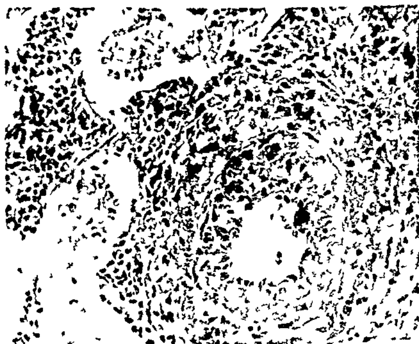


Fig 2 Invasion of the wall of an artery in the lung by Histoplasma The organisms which appear as small rounded dots are intracellular in position

years has greatly increased the significance of a positive tuberculin test (except in the aged and underprivileged)

Frequently the *cases of moderate severity* must be distinguished from viral pneumonia and primary atypical pneumonia and it follows that they must also be distinguished from ornithosis Q fever and toxoplasmosis This can be done only by utilizing available diagnostic methods Acute diffuse interstitial fibrosis of the lungs (Hamman and Rich) beryllium pneumonitis pulmonary alveolar proteinosis and alveolar cell carcinoma may also simulate diffuse histoplasmosis In a few cases middle lobe syndrome has resulted from the pressure of histoplasmic tracheobronchial lymph adenitis

Cases with prolonged or intermittent fever accompanied by radiologic evidence of definite pulmonary involvement are most often confused with pulmonary tuberculosis A number of careful studies have shown that in nearly all tuberculosis sanatoriums at

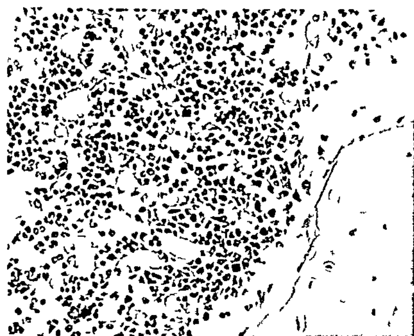


Fig 5 From a section of bone marrow in a case of generalized infection The marrow consists largely of Histoplasma laden phagocyte and undifferentiated cells

lesion diagnostic segmental resection is carried out without delay (10) Several studies indicate that roughly half of these coin lesions turn out to be tumors (primary or metastatic carcinoma for the most part) while the other half are granulomatous lesions (10) In about half of these granulomatous lesions the etiologic agent is identified by culture or by special staining methods The recognized etiologic agents include M tuberculosis Histoplasma capsulatum Coccidioides immitis Blastomyces dermatitidis Nocardia and Cryptococcus neoformans Histoplasma capsulatum is of course particularly common in the mid western United States and geographical factors as well as the results of immunologic tests should always be considered in the preliminary differential diagnosis In the remaining half of the granulomatous lesions no specified etiologic agent is discovered many of these may represent healed mycotic infec



Fig 4 A large pseudo tubercle in the liver composed of epithelioid cells with a peripheral collar of lymphocytes No giant cells are present and organisms were not found in the liver

diagnosis is not made until the segment of lung tissue involved has been removed surgically and studied microscopically and by cultural methods In cases where both tubercle bacilli and fungi are present it is often impossible to decide which agent first gained entry to the lung tissue and which one played the more important role Chronic cystic disease of the lungs and Klebsiella infection with cavitation were the original diagnosis in 2 reported cases Fungal infections other than histoplasmosis bronchiectasis and silicosis may also cause cavitation

The Coin Lesion A significant contribution to our knowledge of histoplasmosis of the lungs has been made by the pulmonary surgeons Watchful waiting is currently frowned upon for patients who show radiologic evidence of an unexplained round or coin

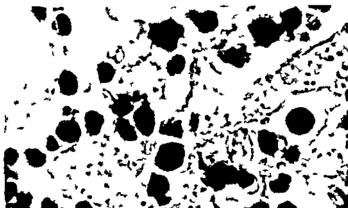


Fig 7 High power of lymph node showing organisms in swollen reticulum cells

Fig 8 Portal lymphocytic infiltration giving picture of lymphatic leukemia in a case with massive bone marrow involvement

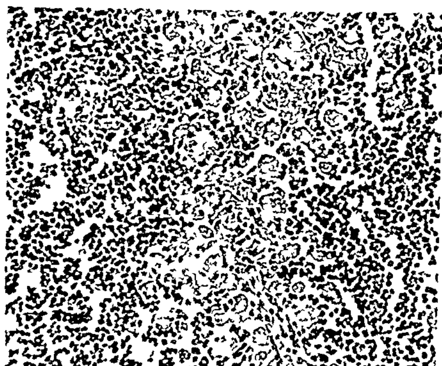


Fig 6 Low power appearance of a greatly enlarged lymph node showing many pale Histoplasma laden cells of reticuloendothelial origin

tions with disappearance of the organisms Some of the coin lesions turn out to be lipoid pneumonia (usually from mineral oil aspiration) unresolved pneumonia silico tuberculosis infarcts benign pulmonary tumors etc

Recent studies (11 12) indicate that the Grocott stain a modification of the methenamine silver nitrate technique of Gomori is much more effective than PAS methods for demonstrating fungi in resected solitary granulomas In a re study of 26 cases previously diagnosed as granulomas of undetermined origin Greendyke and Emerson (11) found histoplasma in 20 cases These studies were made in a non endemic area Of the 20 cases 13 were asymptomatic and discovered by routine out patient chest films or incidentally to hospitalization for other reasons

Schulz *et al* (12) studied 44 granulomatous lesions resected without pre-operative diagnosis Eighteen were proven to be



Fig 10 Flask shaped ulcer in the colon in a case of the intestinal type

example) should also be given due weight Only when all of these factors have been evaluated can we consider that a coin lesion is unexplained In a young person who does not smoke and whose lesion shows calcification resection is probably inadvisable (12)

Looking at the problem broadly it is clear (although statistical studies rarely face this problem squarely) that at least 10% of surgically resected coin lesions are so innocuous that the surgeon is made temporarily unhappy by the pathological report His thoughts soon turn however to previous and apparently identical cases in which the report showed operable pulmonary carcinoma and he does his own mental mathematics even though the rules for this type of mathematics have not been rigidly formulated The ideal of coin lesion resection without regrets will for sometime remain unattainable but in the meantime every possible effort

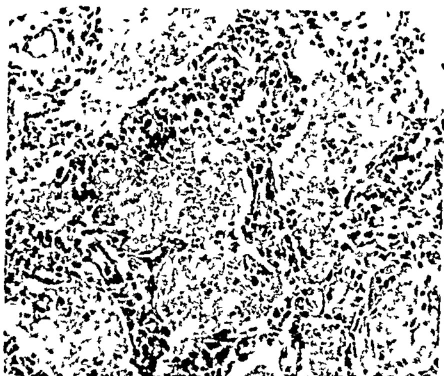


Fig 9 Interstitial pneumonitis in systemic histoplasmosis Large phagocytic cells laden with organisms crowd the alveolar walls and lie free within alveoli

tuberculosis 22 fungal infection (of which 16 were histoplasmosis) 2 sarcoidosis 2 eosinophilic granuloma in 4 cases the etiologic agent was not determined

Few would disagree with the generally held belief that unexplained round or coin lesions should be resected It would seem wise however to treat each case as an individual problem to use all available laboratory tests including cytological study of the sputum for malignant cells and to take into consideration such factors as age sex geographical data (particularly recent migration from a non endemic to an endemic area) history of recent or past pneumonia in the patient and in other members of his family and exposure to dried chicken manure silo dust cave dust decayed wood or other possible sources of spore inhalation The possibility of aspiration pneumonia (during an episode of vomiting for



Fig 19 Lymph node with histoplasmosis Necrosis and calcium deposition is shown Many organisms are still present

diagnosis is that of any subacute illness associated with fever splenomegaly hepatomegaly lymph node enlargement anemia leucopenia anorexia and loss of weight (13) Diagnosis can be made by demonstrating the highly characteristic fungi in the blood bone marrow or biopsy material from lymph nodes liver etc Immunologic tests are of relatively little value in acute fulminating systemic histoplasmosis Lethal disseminated infection is more common in children than in the older age groups

LOCALIZED HISTOPLASMOSIS

Although chronic focal histoplasmosis is most common in the lungs it may occur in practically any organ or tissue in the body and may mimic primary disease of many different organs (13) In its manifold focal forms it is usually difficult and often impossible to establish the diagnosis but certain important points should be kept in mind



Fig 11 Caseation necrosis in the adrenal Complete necrosis in upper right corner and essentially normal adrenal tissue in lower left corner

should be made to improve diagnostic accuracy and to establish broad policies on the democratic basis of free discussion

GENERALIZED HISTOPLASMOSIS

Histoplasma organisms may be found in the circulating blood or bone marrow in cases of pulmonary histoplasmosis which end with complete clinical recovery Mycethemia then does not necessarily indicate that disseminated lesions will occur but gives a clue to the occurrence of the acute fulminating form of the disease as well as the more chronic but progressive types in which one or more organs are conspicuously involved by necrotizing lesions

Generalized or systemic histoplasmosis may occur terminally in patients who have had localizing signs such as pulmonary consolidation diarrhea naso-oral lesions or cardiac murmurs It may also occur without localizing signs in which case the differential

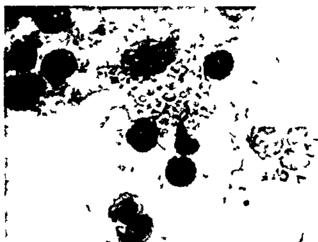


Fig 14 Another infected cell from same preparation as that used for Fig 13

media only by demonstrating the presence of the characteristic intracellular organisms

Cases of ocular involvement simulate tuberculosis and other granulomatous infections so closely that they are rarely diagnosed except in cases of enucleation in which the organisms are readily seen in sections

Vegetative endocarditis is rarely caused by H capsulatum (15) It should be suspected when routine blood cultures are negative for the common bacterial agents and may usually be diagnosed by suitable cultural techniques

Ulcerative Enteritis Here again differentiation of the histoplasmic type from other more common types depends first on thinking of the possibility and secondly on visualizing or culturing the etiologic fungus Histoplasma laden macrophages have been mistaken for erythrocyte laden amoebae (16)

Cerebral histoplasmosis is relatively common in the disseminated form of the disease (17) It may take the form of a localized lesion (histoplasmoma) or may occur as mild or severe meningitis In rare cases it has been established as the cause of acute meningitis by finding organisms in the spinal fluid

Adrenal involvement is very common massive caseous necrosis

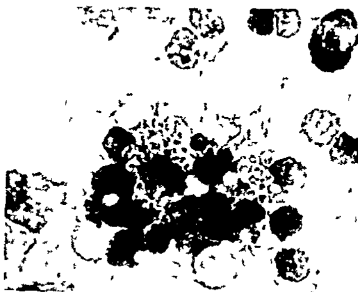


Fig 13 Film preparation from sternal marrow puncture Giemsa stain Two cells containing organisms are shown

Cutaneous and mucocutaneous histoplasmosis must be differentiated from leishmaniasis as well as from other fungal infections (14) This can be done only by identifying the causative organism

Naso oral Type Moore and Jorstad (14) have emphasized the fact that histoplasmosis of the naso oral cavity may simulate rhinoscleroma laryngeal tuberculosis aleukemic leukemia lymphoma leishmaniasis noma blastomycosis and syphilis There is usually little difficulty in establishing the diagnosis by demonstrating the specific organisms

Of particular importance is the differentiation from squamous cell carcinoma especially when nodular and ulcerative lesions of the tongue are present These lingual lesions often simulate carcinoma not only grossly but to some extent microscopically because of atypical epithelial proliferation in the overlying epidermis In studying doubtful biopsy specimens pathologists have learned to look carefully for fungi in sections specially stained by the PAS and Grocott silver methods

The otitic type can be differentiated from other types of otitis

In a fatal case (case A) reported by Parsons and Zarafonetis (3) numerous tubercle bacilli were present in the walls of a pulmonary cavity and in the kidneys The caseous adrenal glands and the glossal pharyngeal and colonic ulcers contained histoplasma organisms without tubercle bacilli while the widespread miliary tubercles in the liver spleen pituitary thyroid tongue pharynx lungs bronchial lymph nodes kidneys prostate and small intestine showed neither fungi nor acid fast organisms (This report antedated the use of special stains for fungi) In pulmonary cavities tubercle bacilli and histoplasma organisms have been found side by side (21) The author (22) has seen two fatal cases of typical cavitary pulmonary tuberculosis both of which showed early focal lesions with numerous histoplasma organisms in the adrenals the pulmonary cavities showed only acid fast organisms These observations illustrate the difficulty of determining which infection is primary and which is secondary

The evidence suggests that neither the fungus nor the tubercle bacillus exerts any demonstrable inhibitory action on the other It is possible that impairment of the immunologic machinery by either infection may favor the development of the other Zimmerman (23) believed that the disseminated form of histoplasmosis was more likely to occur in patients with tuberculosis It is perhaps even more probable that some unknown biochemical lesion (enzymatic abnormality) may critically lower the resistance to both organisms simultaneously The unmasking of such a basic mechanism might prove more important than the discovery of new antibiotics since there is reason to believe that it may be impossible to treat the two infections simultaneously and effectively

The occurrence of *dual fungal infections* is not surprising in view of the ubiquity of pathogenic fungi and the infrequency with which they cause clinically important tissue destruction Here again a deteriorating immunologic mechanism may cause flaring up of two or more quiescent infections In two fatal cases histoplasmosis cryptococcosis tuberculosis and Hodgkin s disease have all been found at autopsy

Malignant Lymphoma Phelps and Mallory (24) reported pulmonary histoplasmosis in a patient dying with primary carcinoma

with great enlargement of the adrenals is the usual picture (18 19) Differential diagnosis from other types of Addison s disease must occasionally be considered, when other localizing signs are absent Theoretically with massive and partly calcified lesions demonstrable radiologically needle punch biopsy would establish the diagnosis since the caseous masses contain tremendous numbers of fungi demonstrable by special stains (see Figs 1 and 11)

Other localized types of histoplasmosis such as joint involvement renal and prostatic involvement present special problems and can be diagnosed only by demonstrating the organisms

DISEASES ASSOCIATED WITH HISTOPLASMOSIS

It has become clear in recent years that several disease entities and syndromes occur in association with histoplasmosis much more frequently than could be explained by chance alone

The *specific infections* most often associated with histoplasmosis are tuberculosis and a variety of fungal infections other than histoplasmosis notably cryptococcosis Active pulmonary tuberculosis is found at autopsy in 5 10% of all fatal cases of histoplasmosis (Hodgson *et al* 21) In surgical material Schulz *et al* (12) found concurrent fungal and tuberculous infection in 4 of 140 specimens Since this dual infection is rarely if ever recognized during life time relationships are difficult to establish It is possible that a tuberculous pulmonary cavity may serve as the portal of entry for the fungus but cavitary histoplasmosis could equally well be complicated by pulmonary tuberculosis particularly in view of the fact that patients with uncomplicated pulmonary histoplasmosis often reside in tuberculosis sanatoriums where there is a particular danger of acid fast infection

Judging from fatal cases reported it would appear that in cases of dual acid fast and mycotic infection disseminated lesions are much more often the result of histoplasmosis than of tuberculosis That dual infection is not always fatal is illustrated by a case reported by Procknow in which multiple histoplasmic lesions in the lung were superimposed on a quiescent solitary tuberculous lesion the histoplasmic snowstorm cleared after a few months

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inally in patients with blood dyscrasias (27) The widespread replacement of normal reticulo endothelial cells by atypical cells would be expected logically to result in lowered resistance

Regarding the second explanation the author can say only that he has two cases of histoplasmosis and Hodgkin s disease in his files in which there has been serious disagreement among expert morphologists as to whether the lesions of Hodgkin s disease are true or spurious in other words the lesions of Hodgkin s disease are not sufficiently typical to result in uniformity of opinion More over as the author (13) pointed out in 1949 in the reported cases of histoplasmosis associated with leukemia the leukemia was not entirely convincing and the possibility of a leukemoid reaction to bone marrow injury by the fungi could not be excluded In the more recently reported cases (25) there appear to be similar devia tions from the typical picture of leukemia and lymphoma In two cases for example biopsied lymph nodes were diagnosed confi dently as lymphosarcoma but eventual postmortem examination showed no evidence of any type of lymphoma In one of these cases the reporters believed that there had probably been a mix up in the original biopsy slide but in the other case no such explanation was suggested One must conclude then that the histoplasma infection had either cured the lymphosarcoma or had simulated lymphosar coma so closely that experienced observers were misled Even the two cases of leukemia reported by Nelson *et al* (25) were aleukemic and somewhat lacking in documentation This view is comparable to the recent recognition of the difficulties in sharply separating such conditions as myeloid metaplasia from leukemia In short the author believes that many of the lymphomas and lymphosar comas and some of the cases of atypical Hodgkin s disease associated with histoplasmosis are in reality reactive hyperplasia of the reticuloendothelial system

The third possible explanation at least in a modified form has been most eloquently defended by Dubin (26) This worker gives serious consideration to the hypothesis previously advanced by Brandt (28) and by Desjardin (29) that various types of injury to the reticuloendothelial system including chronic infections may initiate Hodgkin s disease Assuming that the primary disturbance

of the liver The neoplastic diseases most often associated with disseminated histoplasmosis however are those which originate from the cells of the reticulo endothelial system particularly lymphatic leukemia Lymphosarcoma and Hodgkin s disease all of which are considered to be varieties of malignant lymphoma

Nelson *et al* (25) recently reviewed 25 cases in which histoplasmosis occurred in association with malignant lymphoma of one or another of the three types Hodgkin s disease predominating Among these 25 cases are included the two previously mentioned in which Hodgkin s disease was associated with histoplasmosis cryptococcosis and tuberculosis

The association of histoplasmosis with lymphoma is too frequent to be explained as purely fortuitous As has been pointed out by several investigators this leaves us three possible explanations (1) malignant lymphoma may so disturb the normal defense mechanisms of the body that exogenous infection or endogenous flaring up may result in progressive disseminated fungal infection (2) under certain immunologic conditions the tissue reactions to the fungi may closely resemble malignant lymphoma and (3) the mechanisms for the development of malignant lymphoma may be set in motion by the presence of the fungi

The first explanation can be supported by considerable evidence and is most generally accepted The author believes on the basis of personal experience that the second explanation is the correct one in certain cases The third explanation is least probable but of greatest interest and the author feels that it would be unscientific to eliminate it without further experimental study

In support of the first explanation an impressive array of evidence can be adduced In Hodgkin s disease (and probably in other lymphomas) there is a demonstrable poverty of immunologic mechanisms (26) A wide variety of organisms have been cultivated from lymph nodes in Hodgkin s disease antibodies against these organisms are present in very low titre or more often are completely absent (26) Zimmerman found malignant lymphoma in association with about one third of a series of 71 cases of disseminated cryptococcosis Disseminated toxoplasmosis and disseminated salivary gland virus infection in adults often appear to occur term

relationships have been variable Histoplasma organisms have been found in the re study of an excised lymph node which originally was diagnosed as sarcoidosis years later the patient died with generalized histoplasmosis and sarcoid lesions In other cases the picture has been that of sarcoidosis but after years of a chronic benign course histoplasma organisms have appeared in excised lymph nodes (33)

The author s view based on studies of several cases is as follows It seems quite possible that infection with H capsulatum may complicate cases of true sarcoidosis On the other hand careful study of several cases which clinically and histologically satisfy all criteria for a diagnosis of sarcoidosis has shown that the entire picture may be explained as a special type of reaction pattern to histoplasma organisms (19) Given a large focus of necrosis with masses of fungi in an organ such as the adrenals dissemination to organs with good immunity and sensitivity to the fungal antigen may result in lesions of the sarcoid type

Histoplasma organisms are probably only a rare cause of the picture known as sarcoidosis Other fungi the tubercle bacillus and probably other undiscovered antigens may stimulate the reticulo endothelial system in such a way as to cause the characteristic lesions and radiologic picture

It is obvious that methods for discovering the etiologic agents of sarcoidosis are of great importance In those cases where the antigen is fungal and live fungi are present somewhere in the body treatment with cortisone may cause flaring and dissemination of the infection unless it can be controlled by some as yet undiscovered antibiotic

Allergic Diseases As suggested above there is reason for believing that sarcoidosis may be an allergic manifestation of histoplasmosis somewhat comparable to the occurrence of erythema nodosum and erythema multiforme in association with coccidiomycosis although the latter are transient Allergic arteritis is common in histoplasmosis particularly in the lungs (34) and in the meninges (17) It seems possible that certain other allergic conditions of currently unknown cause may represent reactions to smouldering histoplasmic and other fungal infections

in this disease may be an enzymatic deficiency resulting in a low level of antibody formation the result of antigenic stimulation would be first proliferation of reticuloendothelial cells and eventually a purposeless multiplication of these cells in a futile attempt to overcome their functional deficiency Certainly if there is truth in this hypothesis H capsulatum an organism which multiplies within reticuloendothelial cells would be a powerful stimulus to the proliferation of these malfunctioning reticulum cells

While it is true that in most cases the disseminated fungal infection appears to occur late in the course of the lymphoma in at least one instance it was present in a cutaneous nodule removed 4 years before death of the patient (30) The apparent absence of fungi in the early stages might be due to their small numbers in a delicately balanced host parasite relationship thus the fungi might act as a constant mild stimulus growing massively in tissues only as a terminal event Admitting the speculative nature of such a theory it deserves at least careful testing by experimental methods

Sarcoidosis is another reticuloendothelial condition (in this case a relatively benign one) which is rather frequently associated with histoplasmosis While some workers feel that it is a specific disease entity others look on it as a reaction pattern resulting from a unique immunologic response of an allergic nature to a variety of antigenic stimulants (31) Still others realizing that non caseating hard tubercles form in response to many different agents make a distinction between the sarcoid lesion and true sarcoidosis (32)

Regardless of what new information further studies may bring to light certain facts at present are clear Hard tubercles resembling those of sarcoidosis occur frequently in the liver spleen and lymph nodes in cases of chronic but eventually fatal histoplasmosis in which only one or two organs show necrotic lesions with demonstrable fungi and fungi are rarely found in these hard tubercles even with special stains (19) In a number of cases lymph node biopsy has led to a confident pathological diagnosis of sarcoidosis months or years later histoplasmosis has been found at autopsy—usually with necrotic foci in one or two organs and sarcoid like lesions in the other viscera and in the lymph nodes (19 30) Time

10 Isaacs H J and Isaacs J H What should we do about isolated lesions of the lung? *M Clin North America 39* 161 1955
11 Greendyke R M and Emerson G L Occurrence of histoplasma in solitary pulmonary nodules in a non endemic area *Am J Clin Path* 9 36 1958
12 Schulz D M Tucker E B and McLoughlin P T Observations on the laboratory diagnosis of granulomatous inflammation of the lungs *Am J Clin Path 29* 28 1958
13 Pinkerton H Histoplasmosis *Advances Int Med 3* 197 1949
14 Moore M and Jorstad L H Histoplasmosis and its importance to otorhinolaryngologists *Ann Otol Rhin & Laryng 52* 179 1943
15 Beamer P R Reinhardt E H and Goodof I I Vegetative endocarditis caused by higher bacteria and fungi review of previous cases and report of 2 cases with autopsies *Am Heart J* 9 99 1945
16 Henderson R G Pinkerton H and Moore L T Histoplasma capsulatum as a cau e of chronic ulcerative enteritis *J.A.M.A 118* 885 1942
17 Shapiro J I Lux J J and Sprofkin B E Histoplasmosis of the central nervous system *Am J Path 31* 319 1955
18 Crispell K R Parson W Hamlin J and Holl f eld G Addison s disease associated with histoplasmosis Report of four cases and review of the literature *Am J Med* 0 3 19 6
19 Pinkerton H and Iverson L Histoplasmos s Three fatal cases with disseminated sarcoid like lesions *A M A Arch Int Med 90* 456 19
0 Hodgson C H Weed L A and Clagett O T Pulmonary histoplasmosis *J.A M A 145* 80 1951
21 Meleney H E Histoplas nos s (reticulo-endothelial cytomycosis) A review *Am J Trop Med 20* 603 1940
22 Stanton M F and Pinkerton H Unpubl shed observations
23 Zimmerman L E Some contributions of the histolog cal method to the study of fungus infect ons *T New York Acad Sc 19* 358 19 7
4 Phelps B M and Mallory F B Tox cirrhosis and primary liver cell carcinoma complicated by histoplasmo is of the lung Fifteenth annual report of the med cal department of the United Fru t Co New York Un ted Fru t Company 19 6 p 115
5 Nelson N A Goodman H L and Oster H L The associat on of h stoplasmos with lymphoma *Am J M Sc 233* 56 19 7
6 Dubin I N The poverty of immunological mechan sms n patients with Hodgkin s disea e *Ann Int Med* 27 898 1947
27 Hem ath F A and Pinkerton H Disseminated cytomegal c inclus on d sease and disseminated toxoplasmos s in an adult with myelo d metapl sia *Am J Cl n Path 26* 36 19 6
28 Brandt M Beitrag zur pathologischen Anatom e der Lymph ogranulomatose *Arch Path An t* 7 400 19 9
9 Desjardins A U The et logy of lymphobla toma *J.A.M.A 103* 1033 1934
30 Re mann H A and Price A H H stoplasmosis n Pennsylvania Confusion w th sa coidos and expe mental ther py with b cillomycin *Pennsylvania M J 52* 367 1949
31 Te lum G Aller c hyperglobulino s and hyal nos s (paramylo dos s) in the reticulo endothel al system n Boe k s sarco d and other condit ons morpholog c immunity patt rn *Am J Path* 4 389 1948

Healed Histoplasmosis Splenic calcification is probably often the result of healed histoplasmosis (35) and the author has suggested that the peculiar type of splenomegaly known as Stengel Wolbach sclerosis may have a similar origin (19)

A study of fatal cases of histoplasmosis often shows scarring of the liver kidneys lungs and other organs (19) Since there is evidence that fungal lesions even when markedly destructive to tissues may eventually heal completely it seems possible that certain cases of diffuse and focal fibrosis in various organs may represent healed fungal lesions If so proof will be very difficult but the author feels that it is the group of granulomatous and fibrotic conditions of currently unknown etiology that one must suspect histoplasma as a causative agent

The accurate differential diagnosis of histoplasmosis the explanation of its frequent association with apparently unrelated conditions and the discovery of new clinico pathological manifestations of the infection (particularly when fungi have largely or entirely disappeared from lesions) will continue to challenge the ingenuity of clinical and experimental investigators for many years to come

REFERENCES

1 Furcolow M L Schwarz J Howell B and Grayston J T Incidence of tuberculin histoplasmin and blastomycin reactors among a group of school children *Am J Pub Health 43* 1523 1953

2 Baum G L and Schwarz J Pulmonary histoplasmosis *New England J Med 258* 677 1958

3 Parsons R J and Zarafonetis C J D Histoplasmosis in man report of 7 cases and a review of 71 cases *Arch Int Med 75* 1 1945

4 Schwarz J. The pathogenesis of histoplasmosis *Tr New York Acad Sc 20* 541 1958

5 Christie A and Peterson J C Pulmonary calcification in negative reactors to tuberculin *Am J Pub Health 35* 1131 1945

6 Straub M and Schwarz J The healed primary complex in histoplasmosis *Am J Clin Path 25* 727 1955

7 Furcolow M L Clinical diagnosis of histoplasmosis *Proc of the Conference on Histoplasmosis 1952* Pub Health Monogr No 39 1956 p 3

8 Puckett T F Pulmonary histoplasmosis *Am Rev Tuberc 64* 455 1952

9 Loosli C G Histoplasmosis Some clinical epidemiological and laboratory aspects *M Clin North America 39* 171 1955

MEDICAL MANAGEMENT OF HISTOPLASMOSIS

J Lewis Yates H Vernon Langeluttig and C A Brasher

INTRODUCTION

The need for effective medical management of histoplasmosis cannot be denied A completely satisfactory or well standardized method of management of the disease has not been developed at this time Some of the confusion which exists as to the effectiveness of the presently available therapy may be explained by the following (1) Histoplasmosis presents varied and incompletely understood clinical pictures (2) There is much variance in terminology (3) Many patients with benign disease do not consult a physician Whether they do or not they are very likely to improve spontaneously without specific therapy or in spite of the type of treatment used (4) Serious types of disease may fluctuate in activity and become inactive spontaneously or have periods of clinical and x ray remission without complete arrest of the disease (3 4 7 17 18 26 29)

The fact that many people in endemic areas have benign types of disease virtually without realizing it is shown by the finding that 75 to 90% of this population are positive histoplasmin reactors without a specific diagnosis of active histoplasmosis having been made previously (4 7 8 24) A significant percentage of these individuals eventually develop important disease as shown by the finding that 7% of admissions to sanatoria in the endemic area have clinically significant histoplasmosis (24)

AIMS OF MEDICAL MANAGEMENT

It may seem superfluous to discuss the aims of medical therapy but this will assist in crystallizing our thinking The aims of therapy will vary with the type of histoplasmosis to be treated Unnecessary drug therapy should be avoided until the development of a cheap

32 Freimann D G Sarcoidosis *New England M J 239* 664 1948

33 Symmers W S A case of histoplasmic lymphadenitis following recovery from sarcoidosis *Brit M J 4996* 786 1956

34 Furcolow M L Further observations on histoplasmosis *Pub Health Rep 65* 965 1950

35 Schwarz J Silverman F N Adriano S M Straub M and Levine S Relation of splenic calcification to histoplasmosis *New England J Med 252* 887 1955

NON SPECIFIC MEDICAL THERAPY AND REST

Many of the principles of therapy discovered to be of value in other infections notably tuberculosis can be applied to the therapy of histoplasmosis (3 52) Supportive treatment and measures to assure patient comfort such as antipyretics et cetera so long the cornerstone of therapy in tuberculosis can be used to good advan tage This is especially true of patients with acute benign pul monary histoplasmosis Because of some danger of serious extra pulmonary dissemination or the development of progressive pulmonary disease it behooves the physician to advise a definite rest program It is true we do not know how often dissemination or development of progressive pulmonary disease occurs in patients with benign histoplasmosis but the incidence of all types of histo-plasmosis in the endemic areas is so great (75 to 90% of the population) that any one physician is likely to see a moderate num ber of patients with serious types of histoplasmosis Since there seems to be an increased incidence of thrombophlebitis associated with disseminated histoplasmosis a rest program should be modi fied by allowing bathroom privileges and other mild activity to promote muscle tone and improve venous return (3 52) This complication should be borne in mind and when it occurs proper therapy should be instituted promptly Elderly individuals are more likely to develop this complication than patients in the younger age groups

Adequate nutrition is essential and indeed in many of the young people seen with so called benign pulmonary dissemina tion and chest x rays suggestive of miliary tuberculosis this seems to be very important The general nutritional principles found of value in the therapy of tuberculosis are applicable in this disease namely the maintenance of positive nitrogen balance adequate calcium balance and an adequate vitamin intake

At the Missouri State Sanatorium and in most sanatoria where histoplasmosis is being diagnosed at this time the majority of our cases are of the chronic progressive pulmonary type (18 24) The rest program is adjusted according to the severity of the illness as judged by toxicity and clinical progress Seriously ill patients are kept at complete bed rest until their fever has subsided and their

non toxic promptly effective anti fungal agent or agents which may be administered easily preferably by the oral route Since many patients with active benign histoplasmosis will become well spontaneously or with supportive management only (10) judgment must be exercised in order to avoid unnecessary therapy and yet not fail to treat the progressive types of disease adequately One goal should be to save as much useful lung tissue as possible Since 80% of 30 cases with proved chronic pulmonary histoplasmosis have been shown to worsen significantly by x ray over a three year period the indication for specific antifungal therapy depends primarily on making the diagnosis in this type of disease (51) The aim of any therapy is to return an individual to a useful and comfortable active life whenever possible A long course of unpleasant and possibly toxic treatment should not be undertaken if the diagnosis is questionable or if the patient is so aged or has other disease of such a nature as to preclude a successful result Occasionally it is advisable to direct therapy only at the relief of symptoms

Another aim of medical therapy will be to prepare patients for definitive surgery if this may be offered to lessen chances of recurrences after surgery and to decrease complications during the post operative period

The aims in management of patients with progressive disseminated histoplasmosis are first to save life and then to restore them to useful and comfortable living All supportive measures available should be employed as well as the best antifungal agents Even a toxic drug will be used with a calculated risk in those cases with a uniformly grave prognosis Some of these patients may die after a relatively short (3 to 4 week) illness (55) These aims are quite clear when the progressive pulmonary disease or disseminated histoplasmosis are considered However the issues are not nearly as clear with benign pulmonary histoplasmosis There is considerable disagreement as to when to start treatment as to the duration of an initial observation period before starting treatment and whether any therapy at all is advisable (1 3 4 7 18 26 29)

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general condition has improved sufficiently to allow mild activity including bathroom privileges Patients who are not very ill and who are doing well are allowed up for one to two hours at meal times may attend weekly movies do handicraft or go to school part time By allowing the patient these moderate privileges we believe that he is more willing to accept specific therapy which is likely to require several months of hospitalization Of course the optimum length of hospitalization and duration of chemotherapy have not been determined at this time

It seems of the utmost importance to prescribe a well rounded therapeutic program including modified bed rest proper nutrition rehabilitation procedures as well as specific antifungal agents for all patients with active histoplasmosis of a serious nature It is probable that until more adequate antifungal therapy is devised we should use rest principles similar to those used for tuberculosis before the present chemotherapy for tuberculosis was established It can be shown that rest and good nutrition alone will cause temporary remission clinically and by x ray and by sputum examination and in a rare case will result in apparent inactivity of the chronic pulmonary type of disease

An example of this is shown in Case I a 36 year old white male who was admitted to the Sanatorium because of weight loss cough and weakness of three months duration

The physical findings were normal The tuberculin skin test was negative and the histoplasmin skin test was positive The histoplasmin complement fixation was positive with a titer of 1 64 A bronchoscopy was negative A scalene lymph node biopsy revealed chronic granulomatous lymphadenitis Routine blood counts urinalysis and liver function tests were normal and remained unchanged One sputum culture was positive for Histoplasma capsulatum The patient received ten days of oral Amphotericin B therapy prior to leaving the hospital against medical advice We now know that this was ineffective therapy As may be seen (Fig 1) the x rays on admission revealed multiple cavities and infiltrations occupying both upper lung fields Fourteen months later a chest x ray showed practically complete clearing of the disease The patient had not received any specific therapy meanwhile except for a modified rest program at home He has been working as an accountant for approximately one

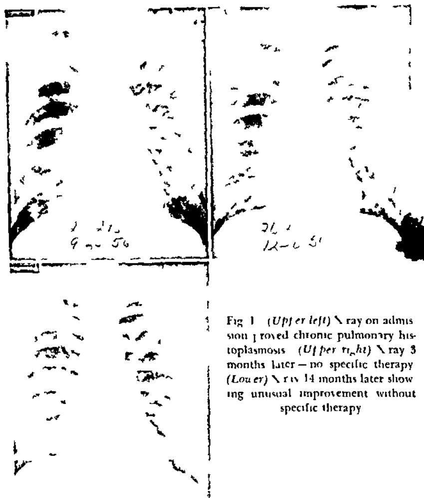


Fig 1 (*Upper left*) X ray on admission proved chronic pulmonary histoplasmosis (*Upper right*) X ray 3 months later — no specific therapy (*Lower*) X ray 14 months later showing unusual improvement without specific therapy

year and he remains clinically well Whether the disease will exacerbate remains to be seen as there are x ray abnormalities remaining at the left apex

This improvement on rest and good nutrition is also illustrated by Case 2 This 35 year old white male had been relatively well until February 1956 when he developed flu with weakness non productive cough and low grade fever which did not seem to respond to the usual antibiotics He lost approxi

mately 15 pounds in weight Two weeks prior to admission he developed right anterior pleuritic chest pain

Physical examination on admission on September 11 1956 revealed a thin white male appearing chronically ill with diminished breath sounds over the right chest The examination was otherwise normal

The important laboratory work revealed a negative PPD skin test a positive histoplasmin skin test and a positive complement fixation at a titer of 1 64 Numerous sputum cultures for acid fast bacilli were negative Four sputums were positive on culture for Histoplasma capsulatum

Course During hospitalization the patient was afebrile except for temperature of 102°F on two occasions during amphotericin therapy Oral Amphotericin B was begun at 6 gm daily on December 8 1956 and continued until February 19 1957 Meanwhile intravenous Amphotericin B was begun on December 13 1956 and continued until March 6 1957 A total of 1 840 mg was given in the three months period The average dose was 60 mg three times a week During the administration of the drug the patient showed moderate side effects such as anorexia headache and nausea but these symptoms were well controlled by analgesics and chlorpromazine Repeated blood counts urinalyses and liver function tests did not show abnormalities The blood urea nitrogen rose no higher than 18 mg during intravenous Amphotericin B therapy On March 8 1957 the patient left the hospital against medical advice having been advised to have surgery On May 22 1957 he underwent a right upper lobectomy at the Mayo Clinic Cultures from the resected tissue were reported negative for Histoplasma capsulatum and acid fast bacilli but there was a necrotic granulomatous type of disease present

The patient s progress since then has been excellent The last chest x ray was taken here on May 14 1958 and reveals no evidence of active pulmonary disease

This patient improved at first on a modified rest program and good nutrition as shown by the definite improvement between the x rays of September 11 and October 29 1956 (see Fig 2) The x ray findings remained stable until Amphotericin B therapy and then seemed to improve further Six sputum cultures collected after starting Amphotericin B were negative for

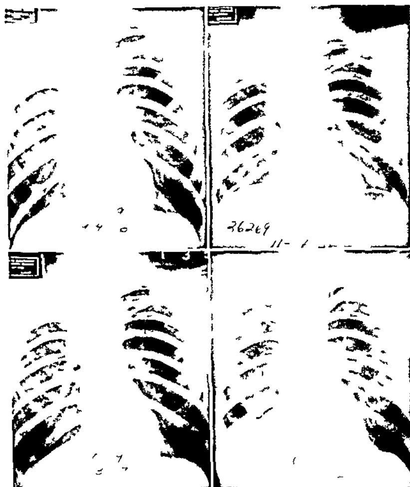


Fig 2 *(Upper left)* X ray on admission—culturally proved chronic pulmonary histoplasmosis *(Upper right)* 71 days later on bed rest and good nutrition alone *(Lower left)* After 5 weeks of I V Amphotericin B *(Lower right)* 1 year after right upper lobectomy—clinically well

Histoplasma capsulatum Symptoms improved but complete cure was not achieved without resectional surgery

INDICATIONS FOR SPECIFIC THERAPY

It is advisable that an observation period of variable duration be considered to observe a diagnosed or a suspected case before specific therapy is instituted Specific antifungal agents should be applied in the treatment of (1) persistently symptomatic primary pulmonary infiltrations (2) chronic progressive pulmonary histoplasmosis (3) disseminated histoplasmosis (4) mucocutaneous lesions (which usually indicate disseminated disease) (5) active histoplasmosis superimposed on other chronic debilitating diseases (1 3 4 7 51 52) It is hoped that an inexpensive non toxic oral antifungal agent will be found which will be at least as effective against the Histoplasma Capsulatum as those available for the treatment of tuberculosis Extreme care must be exerted to avoid attributing a favorable result to a medication when the improvement noted may have been due to a spontaneous remission which may occur during the natural course of the disease This is exemplified by the fact that many drugs reported favorably initially have not proven effective after complete studies and adequate follow up evaluations Temporary clinical x ray and sputum improvement may be observed with bed rest and an improved nutritional program (as may often occur in tuberculosis) and indeed sometimes without any therapy at all (see Cases 1 and 2) Patients who consult physicians relatively late with progressive pulmonary disease or disseminated histoplasmosis on a rapid downhill course should be treated immediately with the best antifungal agents available Patients with acute benign histoplasmosis often will show definite improvement in three to four weeks or sooner on a supportive program If signs of dissemination or local progression occur in a diagnosed case specific antifungal therapy should be instituted Signs and symptoms of progression which should indicate immediate specific chemotherapy are persistent and increasing weakness malaise fever weight loss hepatomegaly splenomegaly anemia leukopenia lymphadenopathy increasing or obviously active x ray findings mucocutaneous ulcers severe cough and hemoptysis

pleurisy associated with other indications of activity in a known case and most important of all persistently positive cultures of the sputum the bone marrow and the blood

Although a certain number of disseminated cases will become well eventually without drug treatment (51) it is better not to gamble with a possibly unfavorable result Furthermore the shortening of a patient s illness is of definite value providing the therapeutic agent is not dangerous The toxic effects of an agent should be carefully evaluated and balanced continuously against any benefits which may accrue To be convincing the beneficial effects should begin in a relatively short time such as three to six weeks depending on the type of disease

In the evaluation of an antifungal agent it is advisable to restrict the types of patients treated to those with histoplasmosis proven by mycological means such as positive sputum or gastric cultures positive bone marrow cultures or positive methenamine silver stains from resected tissues However we believe that it is important to treat with antifungal agents those excessively toxic patients who can be considered to have active histoplasmosis showing progression and who have been diagnosed on the basis of x ray findings clinical picture positive skin test and serological findings but without having proved histoplasmosis on a cultural or histological basis (24) A similar principle has been followed in the treatment of tuberculosis particularly in young persons with disease that is apparently active by x ray who have positive skin tests and who have a history of contact with individuals with active tuberculosis

THERAPEUTIC AGENTS AVAILABLE

Numerous agents have been proposed for use as effective antifungal drugs A brief review of many of these which have been studied follows In 1945 Elson (56) reported that certain of the diamines exerted a fungistatic effect on blastomyces dermatitidis so these were tried on Histoplasma capsulatum but the in vitro studies with stilbamidine and hydroxystilbamidine demonstrated that concentrations required to inhibit Histoplasma capsulatum could not be achieved safely in vivo This has been confirmed by clinical trials

in cavitary pulmonary histoplasmosis (6 19) Aminostilbamidine has failed in clinical trials Beta diethylaminofencolate (MRD 112 Merrell) has proved disappointing, although there are some reports in the literature of beneficial effect in pulmonary histoplasmosis (1 3 4 19 23) Ethyl vanillate has a narrow therapeutic index and gastric irritation frequently occurs at the effective level of between 20 and 30 mg per cent (1 3 26) Nystatin is absorbed poorly from the gastrointestinal tract and produces intolerable side effects when given intravenously although animal studies revealed it was an effective in vivo antifungal agent (3 14 15 16 23) It has been used successfully in local injection for mucocutaneous ulcers and also as a topical agent Experience at the Missouri State Sanatorium failed to prove that it had any demonstrable effect on chronic cavitary histoplasmosis (23) Amebacide (Rx Lilly) is shown to be active in vitro but has proved ineffective in clinical trials probably due to the fact that it is poorly soluble in water (4)

Sulfapyridine sulfathiazole and sulfadiazine have been shown to protect and cure mice challenged with Histoplasma Capsulatum infection (20) Combining sulfadiazine with nystatin or Amphotericin B produced better results in histoplasmosis in hamsters than when either drug was given alone (2, 4 8) However the fungistatic action demonstrated in the laboratory has not been borne out in most clinical trials when these sulfas were used alone (3 23 25) Further clinical tests combining sulfonamides with more active agents such as Amphotericin B are definitely indicated since Christie suggests that they are beneficial in disseminated disease in children (40 53) Actidione (cycloheximide) has not proven effective clinically Candicidin was found to be too toxic for human use as was ascosin An antibiotic Nepera 1968 seems helpful in mice is effective in vitro and warrants clinical trials (44 45) However this drug is apparently not being produced at this time in quantities sufficient for trials as a systemic medication

Various sex hormones do show in vitro fungistatic activity Studies in animals and humans are too few for evaluation at the present time (1 11) Disulferam (antabuse) shows in vitro activity but is of no value clinically (4 28) Numerous other chemical compounds have been tested unsuccessfully either because they

proved ineffective or too toxic Some of these are Iodides thymol quinine aminosalicylic acid quinacrine urethane aminopterin neoarsphenamin stilbophen stilbamine vitamin K and antihista mines (1 3 1 21) Isoniazid can be shown to have some in vitro effect as well but our experience in treating patients with com bined tuberculosis and histoplasmosis does not indicate that it is of definite clinical value in histoplasmosis

Broad spectrum antibiotics and even penicillin have been used by us and others in certain cases of the chronic pulmonary disease with apparent clinical improvement However we have not been able to demonstrate conversion of sputum and believe this clinical improvement is due to the inhibition of secondary bacterial in vaders in the large cavities which are present in chronic pulmonary histoplasmosis

ACTH and adrenosteroids have been reported of value in some disseminated cases but were of little value in mice (4 26) Disseminated histoplasmosis not infrequently involves the adrenal cortex producing clinical adrenal insufficiency (52 see Case 4) This must be kept in mind in evaluating these results since spon taneous improvement in disseminated cases has been observed (51) Steroids may serve to tip the balance favorably in borderline cases Critical judgment must be used in evaluating results in a spon taneously remitting disease The use of adrenosteroids in active chronic pulmonary histoplasmosis without effective antifungal therapy should be considered dangerous

Pimaricine (Lilly) an antibiotic of the polyene type has shown antifungal activity in in vitro studies roughly comparable to that of Amphotericin B and nystatin It has been prepared as an oral drug but causes considerable gastrointestinal reaction which pre vents most patients from taking it well It may prove effective in a parenteral form but is not available as yet (5 49)

AMPHOTERICIN B*

Much of the discussion in this section is based on our personal experience

Amphotericin B seems to show promise as a broad spectrum antifungal agent (3 41 42 52) It is an amphoteric antibiotic derived from a previously undescribed species of Streptomyces iso

in cavitary pulmonary histoplasmosis (6 19) Aminostilbamidine has failed in clinical trials Beta diethylaminofencolate (MRD 112 Merrell) has proved disappointing although there are some reports in the literature of beneficial effects in pulmonary histoplasmosis (1 3 4 19 23) Ethyl vanillate has a narrow therapeutic index and gastric irritation frequently occurs at the effective level of between 20 and 30 mg per cent (1 3 26) Nystatin is absorbed poorly from the gastrointestinal tract and produces intolerable side effects when given intravenously although animal studies revealed it was an effective in vivo antifungal agent (3 14 15 16 23) It has been used successfully in local injection for mucocutaneous ulcers and also as a topical agent Experience at the Missouri State Sanatorium failed to prove that it had any demonstrable effect on chronic cavitary histoplasmosis (23) Amebacide (Rx Lilly) is shown to be active in vitro but has proved ineffective in clinical trials probably due to the fact that it is poorly soluble in water (4)

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ments to tolerance when it may then be given three times a week in full dosage We have not treated anyone with more than 1 mg per kilogram per day

Practically every patient receiving the antibiotic exhibits mild side effects These consist of malaise anorexia nausea low grade fever and headache Tables I and II present the severity frequency and type of side effects observed by us during the therapy of 24 cases of histoplasmosis with intravenous Amphotericin B The majority of patients gradually develop tolerance to fever

TABLE I

SIDE EFFECTS—I V AMPHOTERICIN B

Degree	*Number*	*Per Cent*
None	4	16 6
Mild	6	25.0
Moderate	10	41 6
Severe	4	16 6
Total	24	99 8

TABLE II

SIDE EFFECTS AND RELATIVE FREQUENCY OF SYMPTOMS IN 20 CASES—I V AMPHOTERICIN B

Symptom	*Number*	*Symptom*	*Number*
Anorexia	14	Nausea	5
Chills	1	Diarrhea	1
Headache	9	Rash	1
Fever	8	Pruritus	1
Emesis			

lated from a soil sample from along the Orinoco River in South America Its exact chemical structure is unknown but it is classified as a polyene compound with a molecular formula of C46 H73 NO20 (41) It has no antibacterial activity, but inhibits the growth of yeast and yeast like fungi to a marked degree (35 36 38) Larsh *et al* found 50% inhibition of 5 strains of yeast phase Histoplasma Capsulatum by 0 05 mcg per milliliter concentration using Hela cell techniques The gastrointestinal absorption of this drug is rather poor It is available as a lyophilized yellow powder prepared for parenteral use* (16) The published data on blood urine and spinal fluid levels are meager Bio assay on a few patients indicates definitely inhibitory concentrations in blood There is prolonged excretion in the urine with significant antifungal effects for as long as two months after discontinuance of intravenous therapy (54) This suggests that the drug accumulates in the kidneys and then it is released in the urine gradually This concept is supported by the tendency for the blood urea nitrogen to rise progressively as therapy continues The phenosulfonephthalein and urea clearance tests are progressively depressed also Forcing fluids will tend to decrease the B U N elevation These renal function tests return to normal within a two to four week period after discontinuance of the drug

Increased resistance to Candida species to nystatin and Amphotericin B has been induced in vitro (48) The development of resistance in clinical situations has not been thoroughly investigated as yet

The dosage usually ranges between 0 5 mg and 1 0 mg per kilogram of body weight given intravenously in 500 to 1 000 cc of 5% glucose in water over a six hour period It is given daily to seriously and acutely ill patients and to initiate a course of therapy (3 52) After the dosage has been increased to tolerance or the patient has shown considerable improvement the frequency is usually decreased to three times a week The optimal duration of therapy is unknown The treatment is begun with a 25 mg dose usually given daily and gradually increased by 5 mg incre

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* Each 50 mg of Amphotericin B is packaged with 41 mg of sodium desoxycholate with sodium phosphate as a buffer under the name of Fungizone by Squibb

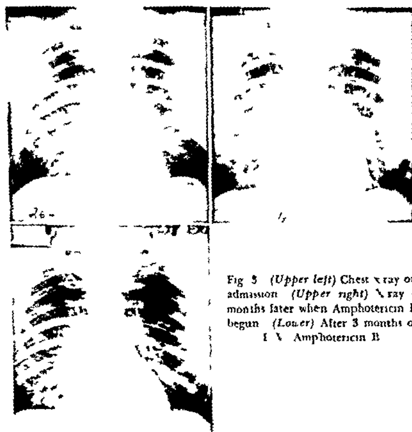


Fig 5 (*Upper left*) Chest x ray on admission (*Upper right*) X ray 4 months later when Amphotericin B begun (*Lower*) After 3 months of I V Amphotericin B

One investigator is administering small doses of hydrocortisone with each treatment and beneficial results are noted in the reduction of side effects (50) This may greatly improve patient acceptance of the drug In an occasional patient phlebitis and/or periphlebitis affecting the vein used for administration may be a problem This usually subsides spontaneously within 24 to 48 hours Avoidance of recently used veins will help

The results of therapy of progressive types of histoplasmosis with Amphotericin B have been favorable As would be expected the results in disseminated histoplasmosis have been more dramatic than with chronic pulmonary disease The problem pre

malaise and headache The nausea is controlled fairly well by chlorpromazine and meclizine and the fever and malaise by salicylates All of the patients with severe side effects were difficult to control by ancillary medications (steroids were not used) The side effects of the drug were poorly controlled with only one of the moderate group Only one patient failed to receive at least six weeks therapy because of side effects Two were discontinued because of elevated B U N or rash Two who needed more therapy left against advice at the end of two months therapy One of these has shown a sputum relapse and the other is lost to follow up Chills will occur in an occasional patient and are helped by adjusting the dosage and time of administration of salicylates by sedation and by keeping the patient warm These side effects do not seem to be serious except that they tend to limit patient acceptance of the therapy This of course will vary considerably from patient to patient and will depend largely on their understanding of the need for treatment and physician patient rapport One of our patients with culturally proved pulmonary histoplasmosis who received great benefit from the treatment took the drug for four months although he spiked temperature of 101° to 102° F every day of therapy in spite of other medications This patient s chest x rays are shown in Fig 3 Note the increase in x ray findings between September 6 1956 and January 10 1957 when Amphotericin B was started This patient remains well and is working full time more than a year after discharge from the hospital The patient acceptance is also influenced adversely by the mode and duration of administration of each dose More serious side effects do occur Azotemia usually of mild degree occurs rather uniformly and is usually accompanied by decreased urea clearance and phenosulfonphthalein excretion The blood urea nitrogen commonly rises to 30 mg per cent and occasionally higher Purpuric eruptions rarely have been observed Pruritus occurs occasionally during the actual administration Temporarily discontinuing the drug for more than 48 hours usually necessitates restarting the drug at a lower dose and reactions which occur are usually more severe than those observed initially Two cases restarted after more than two weeks lay off have presented anaphylactoid type of reactions

was readmitted to the hospital with adreno-cortical insufficiency with an exacerbation of his clinical findings including fever weight loss general malaise splenomegaly and renal histoplasmosis as shown by positive urine cultures He has again responded well to Amphotericin B given along with adrenosteroid therapy

Amphotericin B must be considered an effective drug in the treatment of histoplasmosis It may be considered most effective in disseminated disease and may be used to speed recovery of persistent benign pulmonary histoplasmosis It has proved to be suppressive in chronic progressive pulmonary disease If there is much destruction of lung tissue surgical excision is advisable when feasible (22 24 25 26) Thoracoplasty may be advisable rarely The antibiotic may assist in inactivating disease on one side so that the disease in the other lung may be resected It may be used effectively as an umbrella for resectional surgery of histoplasmosis (22 24 26) The use of Amphotericin B at present may be likened to the early use of streptomycin in tuberculosis This is illustrated by similar uncertainties as to dosage duration of therapy and types of disease for which it should be used However patient acceptance of this therapy is not on a par with that of streptomycin The optimum duration of therapy is unknown but proper evaluation of effectiveness in any case of chronic pulmonary histoplasmosis requires two to four months of therapy At present we are attempting to persuade our patients to accept at least four months of drug therapy X ray improvement frequently does not parallel closely sputum conversion and relief of constitutional and pulmonary symptoms After antibiotic therapy we advise continuation of a modified rest program for six months or longer depending on whether surgery has been done and the amount of disease remaining Extremely severe types of disseminated histoplasmosis such as central nervous system disease (meningitis and involvement of brain tissue) and endocarditis have been observed and should be considered indications for higher dosage and more prolonged therapy with Amphotericin possibly combined with sulfonamides and other antifungal agents (39)

Other drugs are needed and are being tested Short cut meth

sented here is comparable to that seen in moderately and far advanced pulmonary tuberculosis that is if there is considerable destruction of lung tissue (massive necrosis) a morass of diseased tissue is created from which it is most difficult to eradicate the organism without surgical intervention

The character of the sputum usually changes from purulent to mucoid and the quantity is markedly diminished even disappearing in some instances Serial chest x rays reveal clearing of exudative components but cavities and necrotic foci frequently remain although often decreased in size The antibiotic is definitely suppressive and produces remarkable clinical improvement and prompt sputum conversion in most patients This has been shown with 23 of our chronic pulmonary cases in which persistently positive sputum cultures became negative within two to three weeks and remained so as long as therapy was continued Several patients with extensive pulmonary destruction who voluntarily stopped therapy after six to eight weeks have shown sputum relapses One elderly man with a destroyed right lung and a bronchopleural fistula and chronic empyema showed a sputum and clinical relapse within two weeks after cessation of treatment This therapy had been continued steadily for nearly six months This happened in spite of the fact that his x ray had shown absence of empyema fluid and his sputum cultures had been consistently negative for four months Another patient with chronic pulmonary histoplasmosis showed a sputum relapse while we were changing from the insoluble suspension to the lyophilized form which necessitated administration of the drug in lower dosage temporarily He maintained his clinical improvement and underwent a resection without mishap and remains well after one year Another patient with far advanced cavitary pulmonary disease improved by x ray and clinically but his sputum did not convert until after two months of therapy

An elderly man with severe disseminated disease on a down hill course for two months responded clinically within a week and made a remarkable recovery within two months at which time treatment was discontinued because the B U N was 70 mg per cent He remained well for approximately seven months and recently



Fig 4 *(Upper left)* Prior to Amphotericin therapy 5 sputums positive for Histoplasma Capsulatum *(Upper right)* 2 months of I V Amphotericin B Sputums negative for Histoplasma Capsulatum *(Lower left)* 1 month after cessation of Amphotericin B Sputums still negative *(Lower right)* 3 months after stopping Amphotericin B Sputums negative for Histoplasma Capsulatum

ods of testing using Hela cell tissue cultures to determine their effects on yeast forms are being used (5)

Case 4 will illustrate some of the above statements This 57 year old white man with active chronic cavitary pulmonary histoplasmosis was diagnosed on the basis of 5 sputums positive on culture for Histoplasma capsulatum positive histoplasmin skin test positive complement fixation for histoplasmosis and abnormal chest x rays (see Fig 1) Although the tuberculin test was positive 13 sputum cultures were negative for acid fast bacilli

Intravenous Amphotericin B was given for 4½ months During this treatment the patient gained 10 pounds and became afebrile except for a low grade fever occurring on the day of each I V treatment He received 65 mg of Amphotericin B three times a week He suffered moderate side effects including *nausea occasional vomiting intestinal cramping and mild diar* rhea mild chilling anorexia and malaise These were well controlled by salicylates and chlorpromazine The blood urea nitrogen remained normal throughout the therapy The last sputum that was positive for Histoplasma capsulatum was col lected 3 days after starting intravenous Amphotericin B There have been 15 sputum cultures negative since then and 6 of these were collected since Amphotericin B was stopped The patient was readmitted for a short time recently approximately 3 months after stopping Amphotericin B and 3 sputums were negative by culture for Histoplasma capsulatum He suffers infrequent epi sodes of increased cough and purulent sputum with low grade fever and malaise These symptoms are controlled by short courses of broad spectrum antibiotics We believe these are due to secondary bacterial infections of the large cavities remaining in the lungs (see Fig 4)

This patient illustrates the conversion of sputum by Ampho tericin B and the improvement of exudative components of his disease but destructive lung disease remains and it may be only a matter of time until his sputum becomes positive again Surgery is not advisable because of his poor ventilatory function and bilat eral cavitation Perhaps intramuscular Amphotericin B could be used to keep this type of disease quiescent This case is an example of the serious type of chronic pulmonary histoplasmosis and

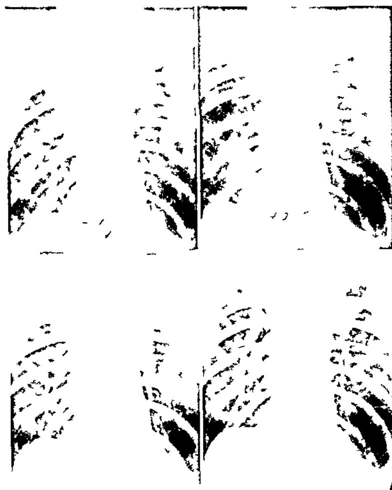


Fig 4 *(Upper left)* Prior to Amphotericin therapy 5 sputums positive for Histoplasma Capsulatum *(Upper right)* 2 months of I V Amphotericin B Sputums negative for Histoplasma Capsulatum *(Lower left)* 1 month after cessation of Amphotericin B Sputums still negative *(Lower right)* 3 months after stopping Amphotericin B Sputums negative for Histoplasma Capsulatum.

illustrates some of the problems which occur in the therapy of this type of disease

SUMMARY

It appears very likely that eventually the therapy of histoplasmosis will include combined drug therapy over longer periods than are used now Perhaps the combination of Amphotericin B and a sulfonamide would be worth a trial at this time Also surgery will be used more freely as a more effective medical therapy evolves

At present Amphotericin B seems to be the best antifungal agent available It is primarily suppressive and has its own toxicity and therefore should be used only where definitely indicated The principles of therapy developed for tuberculosis are useful namely rest adequate nutrition and long combined drug therapy Certainly there is a tremendous need to learn more of the disease itself as well as its treatment This certainly will be forthcoming in the ensuing years as many people are working on this problem Cooperative studies on mycoses such as are being carried out by the British Medical Research Council Veterans Administration and U S Public Health Service on tuberculosis have been inaugurated by the Veterans Administration and U S Public Health Service with regard to these mycoses These should prove to be very fruitful in the future

REFERENCES

1 Procknow J J and Loosli C G Treatment of the deep mycoses *A M A Arch Int Med 101* 6 802 1958
2 Baum G L Schwarz J and Wang C J K Treatment of experimental histoplasmosis with Amphotericin B *A M A Arch Int Med 101* 84 86 1958
3 Tehan P H Yates J L Brasher C A Larsh H W and Furcolo [illegible] L Experiences with the therapy of sixty cases of deep mycotic infection *Dis Chest* 3[illegible] 597 614 1957
4 Silverman F N Schwarz J Lahey M F and Carson R P Histoplasmosis (Review) *Am J Med 19* 410-459 195[illegible]
5 Larsh H W Silberg S L and Hinton A Use of the tissue culture method in evaluating antifungal agents *Antibiotics Annual 1956 1957* Medical Encyclopedia Inc pp 918 922 *Antibiotics Annual 195[illegible] 1958* Medical Encyclopedia Inc pp 988 999
6 Snapper I and Schneierson S S Fungistatic action of stilbamidine and [illegible] hydroxystilbamidine against Histoplasma capsulatum as compared to Blastomyces dermatitidis Pub Health Monogr N[illegible] 3[illegible] 19[illegible] pp 8 11

Conant N F Smith D T Baker R D Callaway J L and Martin D S Histoplasmosis *Manual of Clinical Mycology* Philadelphia Saunders 19 4 Chap 6 pp 119 149

8 Wilson J W *Clinical and Im nunolog c Aspects of F ng s D seas s* Spring field Thoma 1957 Chaps 15 16 pp 143 169

9 Wilson J W Possible approaches to the therapy of fungus diseases In Stern berg and Newcomer *The apy of Fungus Diseases* Boston Little 19 5 pp 25 30

10 McV ckar D L An evaluation of the lal ratory methods for testing fungicides In Sternberg and Newcomer *Th rapy of Fungus D seases* Boston Little 1955 pp 31 33

11 Mankow k Z T The influence f l or al conditions on experimental fungus infection In Sternberg and Newcomer *Therapy of F g is D seases* Boston Little 19 5 pp 90 99

12 Gottlieb David Filip n an antibiotic inhibiting fungi In Sternberg and New comer *Therapy of Fungu Diseases* Boston Little 19 5 pp 14? 14?

13 Campbell C C Preliminary results with a new ant b ot c 1968 (Nepera) in mice with exper mental histoplasmo is porotrichos s and candidiasis *Ibid* pp 160 163

14 Broun R and Hazen F L Nystatin and Act dione Two ant fungal agents produced by streptomyces Noursee *Ib d* pp 164 194

15 Drouhet E and Schwarz J Evaluation of the action of ny tatin on H topl sma capsulatum i vitro and in hamsters and n ce *Ibid* pp ?38 48

16 Campbell Charlotte C Therapeutic activity of my o tatin in mice infected with Histo Cap C Immit s C Neoformanes C Albican Sp Schenkii *Ibid* pp ?5 2 9

17 Beamer P R Schulz D M Walcher D N and Vellios F Hi toplasmosis pathogenes s and im u ology in relation t th rapy *Ib d* pp ?80 86

18 Brasher C A and Furcolow M L Problems in treatment of chronic histoplas mos s as experie ced in o er ?0 a es *Ib d* pp 87 88

19 Bra her C A and Furcolow M L Tr al ith ? hydroxystilbamidine amino stilbam dine MRD 11 and other agents in pulmonary h stoplasmos s experi ments with more than ?0 cases *Ib d* pp 289 91

0 Mayer R L Konopka E Geft S n l Tan ola J Sulfonamides and experi mental hi topl smo i *Ibid* pp ?9 301 al o *Ant b otics a d Chemoth rapy* 6 15 19 6

1 Pappag ams D and Kobayosh G In tro inh bition of C Immit s by anti h tami ics *Ib d* pp 316 3 0

2? Polk J W Bra her C A d Ca tro J and Buck ngham W W The u g cal treatment of pulmonary h top1 m s s with an evaluat on of MRD 11 as a pos bl adjunct *J Thoracic S g 31* 148 16? 19 6

3 Leh n I H Furcolow M L Brasher C A a d L h H W The ap ut c tr als with new er ant fun l gents *Int l otics A n l 1956 195* Medical Encycl pedia Inc pp 46 469

4 Furcolow M L d Brasher C A Ch o c progress (cavitary) h toplasmo s s as a prol lem n Tb anatorium *A Rev Tuberc & Pulm D 73* 609 619 19 6

25 Polk J W Cubiles J A and Buckingham W W The surgical treatment of chronic progressive pulmonary histoplasmosis *J Thoracic Surg* 34 373 341 1957

26 Sutliff W D Experience with the course and chemotherapy of chronic pulmonary histoplasmosis *Am Rev Tuberc & Pulm Dis* 75 919 920 1957

27 Friedman D and Snapper I Histoplasmosis in Brooklyn *Am J M Sc* 234 435 410 1957

28 Lehan P H Brasher C A Larsh H W and Furcolow M L Evaluation of clinical aids to the diagnosis of chronic progressive cavitary histoplasmosis *Am Rev Tuberc & Pulm Dis* 75 938 948 1957

29 Curry F J and Weir J A Histoplasmosis (A review of 100 consecutively hospitalized patients) *Am Rev Tuberc & Pulm Dis* 77 749 763 1958

30 Puckett T F Pulmonary histoplasmosis A study of 22 cases with identification of H capsulatum in resected lesions *Am Rev Tuberc* 67 453 1953

31 Dutcher J D Young M B Sherman J H Hibbits W and Walters D R Chemical studies on Ampho B *Antibiotics Annual 1956 57* pp 866 869

32 Louria D B Feder N and Emmons C W Ampho B in experimental histoplasmosis and cryptococcosis *Ibid* pp 870 877

33 Baum G L Rubel H and Schwarz J Treatment of experimental histoplasmosis *Ibid* pp 878 883

34 Puckett T F Histoplasmosis *Bronchopulmonary Diseases* ed by Naclerio E A Hoeber Harper 1957 Chap 49 pp 408 416

35 Newcomer V D Halde C and Sternberg T H Chemotherapy for coccidioidomycosis in man *Proc of Symposium on Coccidioidomycosis* U S Public Health Service C D C U S Dept of Health Education and Welfare 1957 pp 71 78

36 Fiese M J Therapy of coccidioidomycosis *Ibid* pp 79 82

37 Cohen R Therapy of C immitis infection *Ibid* pp 83 85

38 Littman M L Preliminary observation on I V use of Ampho B in therapy of acute and coccidioidal osteomyelitis *Ibid* pp 86 94

39 Merchant R K Louria D B Geisler I H Edgcomb J H and Utz J P Fungal endocarditis review of literature and report of 3 cases *Ann Int Med* 48 242 266 1958

40 Christie A Histoplasmosis and pulmonary calcification *Ann N Y Acad Sc* 50 1283 1298 1950

41 Sternberg T H Wright E T and Oura M Annals new antifungal antibiotic Amphotericin B *Antibiotics Annual* (Welch & Marti Ibanz) 1955 56 Medical Encyclopedia Inc pp 566 573

42 Steinberg P A *et al* Amphotericin A and B 2 new antifungal antibiotics *Ibid* pp 574 5 8

43 West M K Verwey W F and Miller A K The biologic activity of eulicin *Ibid* pp 231 235

44 Oswald E J Reedy R J and Randall W A An antifungal agent 1968 produced by a new streptomyces species *Ibid* pp 236 239

45 Campbell C C Hill G B and Brook B E Therapeutic activity of a new antibiotic 1968 in mice with experimental histoplasmosis sporotrichosis and moniliasis *Ibid* pp 240 244

46 Bartner E Zinnes H Moe R A and Kulesza J S Studies on a new solubilized preparation of Amphotericin B *Antibiotics Annual* (Welch & Martic Ibanez) *1957 58* Medical Encyclopedia Inc pp 53 58
47 Utz J P Louria D B Feder N Emmons C W and McCullough N B A report of clinical studies on the use of amphotericin in patients with systemic fungal diseases *Ibid* pp 65 70
48 Littman M L Pisano M A and Lancaster R M Induced resistance of Candida species to nystatin and Amphotericin B *Ibid* pp 981 987
49 Struyk A P, Hoette I Drost J Waisvisz J M Van Eek T and Hoogerheide J D Pimaricin a new antifungal antibiotic *Ibid* pp 878 885
50 Seabury J H, and Dascomg H F Experience with Amphotericin B for the treatment of systemic mycosis *Arch I t Med 10* 90C 9¡6 Dec 19ɔ8
51 Ruben H Yates J L Brasher C A and Furcolow M L Course and prognosis of histoplasmo is base l on the analysis of 1 3 proved cases To be published in *Am Jour Med*
5 Yates J L Atay N M Langeluttig H V Brasher C A and Furcolow M L Experiences with Amphotericin B in therapy of 30 cases of histoplasmosis To be published in *D s Cl est*
53 Christie A The d sease spectrum of human histoplasmosis *Ann Int Med 49* 544 555 19 8
54 Vogel R A and Crutcher J C Studies on the bioassay and excretion of Amphotericin B in patients with system c mycoses *Antibiot c Med & Clin Therapy 5* 501 506 19 8
55 Hasty T S Ajello L Wallace G D Howell J and Moore J A small outbreak of histoplasmosis *Am Rev Tuberc & Pulm Dis 78* 576 58? 1958
56 Elson W O The antibacterial and fung static properties of propamidine *J Infect D s* 6 193 194

SURGERY FOR PULMONARY HISTOPLASMOSIS

John W Polk

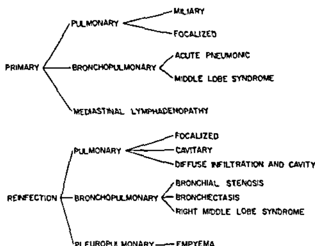
Resection for localized mycotic lesions of the lung has been advocated in the past few years as indicated by several reports in the literature (1 5) These reports deal primarily with the three most important blastomycosis coccidioidomycosis and histoplasmosis although other isolated reports prove that other mycotic lesions may be successfully resected Thirty cases of localized pulmonary histoplasmosis with complete pathological studies have been presented by Forsee (6) and his co workers Chronic progressive histoplasmosis was first described by Johnson and Batson (7) and Bunnell and Furcolow (8) in 1948 Sutliff and his co workers (9) Polk and his co workers (10) and Hughes and his co workers (11) later presented cases comparable with this form of the disease In a more recent report Polk and his co workers (12) demonstrated the wide variety of pulmonary manifestations which may occur with this disease and reported cases which must be differentiated from bronchogenic carcinoma pulmonary tuberculosis bronchiectasis and empyema It is the purpose of this text to consider problems encountered with the surgery of pulmonary histoplasmosis

CLASSIFICATION

The usual classifications of pulmonary histoplasmosis have seemed to be unsatisfactory from a surgical viewpoint and we suggest a modification from pre existing classifications Pulmonary histoplasmosis has complete disregard for tissue barriers and can produce all of the pathological processes encountered with pulmonary tuberculosis That the two diseases occur simultaneously in approximately 20% of our cases seems well documented

Our series of cases includes pulmonary resection of 27 localized lesions histoplasmoma) 29 cases presenting other varying pulmonary pathological processes 12 cases of pulmonary histoplasmosis

in children and approximately 25 cases of histoplasmosis seen at the Missouri State Sanatorium for which no surgery was recommended. In this series the diagnosis has been proven by demonstrating the organism Histoplasma capsulatum in the body tissue or sputum or serologically proven by having a complement fixation titer of 1:8 or higher. No case is included which revealed only a positive skin test. We would therefore present the following classification as a basis of study for the surgeon.



HISTOPLASMOSIS ASSOCIATED WITH PULMONARY TUBERCULOSIS

Indications for Resection

Two considerations of paramount importance must be understood when treating pulmonary histoplasmosis. First, establishing a diagnosis of histoplasmosis may be long and tedious since culture of the organism is not practical in the routine laboratory. Second, if the patient has not localized his disease to a portion of the lung then the prognosis is very poor. If these facts are realized, then surgical resection for histoplasmosis seems to offer excellent therapy in this form of the disease.

The indications for resection of histoplasmosis have not as yet been definitely established due to the paucity of cases. We have therefore utilized many of the established indications for resection

in pulmonary tuberculosis to guide our selection of cases for surgery

PRIMARY HISTOPLASMOSIS

The diagnosis of primary histoplasmosis is often rendered most difficult since the patients with this form of the disease do not develop lesions which cause the organism to be prominent in the sputum Therefore the diagnosis must often be suspected by the history and x ray appearance of the disease and further proof gained by the use of the serological and skin testing studies It is true in this form of the disease that the symptoms may often be so mild that clinical advice is not gained at the time of infection and many late diagnoses are made by the presence of healed calcified lesions in persons with positive histoplasmin skin test and negative tuberculin test It is this group of individuals who in the primary phase of the disease present a miliary involvement of the lungs or a focalized small infiltrative lesion

Other types of the primary phase may occur however such as the acute pneumonic form of the disease or a phase of the disease which we have frequently seen that is right middle lobe syndrome It is our contention that these phases of the disease do require specific treatment

Case Report This 5 year old white female was seen on June 2 1957 complaining of cough aching of the legs and ankles and loss of appetite Her mother stated that she had been running temperature almost every day for one month The child had remained active until the past several days There was no weight loss The child gave a history suggestive of having aspirated material from a rug approximately two weeks prior to this when she had a violent episode of coughing X rays on June 2 revealed an atelectasis of the right middle lobe and some infiltration in the right lower lung field Bronchoscopy was performed at Burge Hospital Springfield Mo June 7 Right middle lobe bronchus was noted to be markedly inflamed and almost completely closed from extensive pressure There was no evidence of any foreign body in either right or left Smears and cultures were made for acid fast organisms These studies were completely negative An intermediate strength tuberculin skin test

was negative The histoplasmin skin test was positive Complement fixation studies were reported positive 1 128

The child was discharged to her home on antibiotics which we felt would help control secondary infection Over the last year the serology has varied from 1 16 to 1 28 On the above program which had been supplemented frequently with broad spectrum antibiotics the atelectasis in the right middle lobe has improved approximately 50% A tonsillectomy was performed July 3 1958 and the child is doing well

Mediastinal lymphadenopathy another phase of the primary form varies in the symptoms that it produces It has been our experience that symptoms do not necessarily follow the preponderance of the adenopathy but seem to vary a great deal with the location of the adenopathy and the resistance of the patient to this disease We have seen patients with extensive bilateral mediastinal adenopathy who presented very few symptoms and in whom the serological titer was not over 1 8 In contrast to this we have seen several cases where the only presenting adenopathy was in the azygos node yet each of these patients presented marked symptoms consisting of malaise fever weight loss and anorexia In both of these patients the serological titer was 1 64 and 1 128 was found Both cases improved after long terms of bed rest and broad spectrum antibiotics which were used to control secondary infection

In two cases the mediastinal node was large and calcified Even though one of these patients had a positive tuberculin skin test and histoplasmin skin test the location of the node in the anterior chest suggested the possibility of a dermoid cyst At the time of surgical removal a diagnosis could not be made until the specimen was opened and studied

RE INFECTION HISTOPLASMOSIS

Re infection histoplasmosis comprises the majority of cases which we have seen who definitely seem to benefit by surgical resection The age group on this type of case in our series has varied from 18 years of age to 68 The various phases of this disease under our classification will be discussed separately

Focalized pulmonary histoplasmosis (histoplasmoma) is being encountered more frequently in surgical resections throughout the

country than any other phase of this disease (11) This is due primarily to the fact that these are coin lesions and exploratory thoracotomies are being done because of the predominating fear of bronchogenic carcinoma Such a surgical attack is definitely warranted The spread of the histoplasmosis from resection of the histoplasmoma is exremely rare In our series of cases and in the series presented by Davis and his group (18) there was no spread of the disease from resection of this type of case Since this case is so fairly common no case report will be given

It is well to bear in mind however that in dealing with this type of case both histoplasmosis and tuberculosis may be found in the same nodule It has therefore been our routine to cover all such patients who are having resection of coin lesions with adequate anti tuberculosis therapy

The large focalized mass seen in two cases diameter over 5 cm must be differentiated from a solid tumor of the lung or bronchogenic carcinoma They frequently have the indefinite borders seen in certain cancers no cavitation is present and in one case the sputum was suspicious for cancer cells even though at the time of surgery no cancer was found At the operating table this type of lesion is firm and with the marked adenopathy present with them suggests that lobectomy should be done prior to biopsy of the lesion

Case Report L B a 59 year old white male was admitted to Missouri State Sanatorium December 12 1956 He stated that he had had severe colds in the winters of 1955 and 1956 and had a productive cough for approximately five months During the summer of 1956 he improved and had no symptoms referable to the respiratory tract In the fall of 1956 he again had severe productive cough and complained of pain in the right chest A roentgenogram revealed a dense mass in the right upper lung field (Fig 1) Following his admission to Missouri State Sanatorium a right upper lobectomy was performed on December 20 1956 The G M S stains of resected lung tissue revealed Histoplasma capsulatum The patient has been followed for 18 months and has returned to his normal work as a farmer (Fig 2 shows a similar lesion)

The third form of disease which we have seen which requires

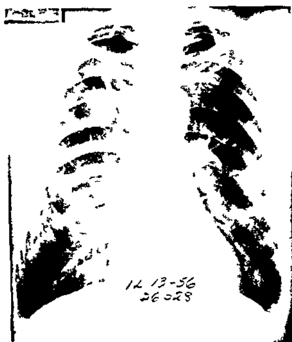


Fig 1

pulmonary resection is the cavitary form of the chronic progressive type This disease has been adequately described by Bunnell and Furcolow (8) and our group (15) If the cavitary component is localized then lobectomy offers excellent results in selected cases

In the cavitary type of this disease which has an extensive diffuse infiltration in both or one lung surgical resection is not recommended until after adequate bed rest and regression of the disease has occurred It has been our opinion from the observation of our own cases and from those in the literature that the spreads following pulmonary resection have occurred in this type of case especially if the resection is carried across diseased portions of the lung

In certain instances the cavitary and infiltrative components of the disease are limited to one lung This type of disease mimics the typically destroyed lung of pulmonary tuberculosis and in our opinion warrants pneumonectomy We will eliminate a case report

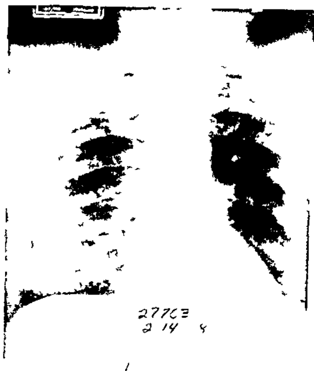


Fig 2

here since this has been shown in previous articles and will present a destroyed lung case with the use of Amphotericin B therapy

In the re infection type of pulmonary histoplasmosis we also have a combination of bronchopulmonary involvement This is a much rarer form of the disease than the pulmonary form which includes the focalized and cavitary process Since this form of disease offers excellent results from pulmonary resection and since histoplasmosis seems to be on the increase and we feel that further such cases will be seen in the future we are including this in the text

True bronchial stenosis as a result of this disease has been demonstrated in only one case in comparison to the numerous cases of bronchial stenosis which have been shown in pulmonary tuberculosis We are unable to explain this pathological phe

nomenon Right upper lobectomy was performed in this case and complete recovery gained

Case Report A K a 39 year old white male was admitted to Burge Hospital Springfield Missouri on October 18 1955 He stated that in September of 1955 he developed a severe cough pain in right chest and sensation of pressure in the right chest He continued to work until October 15 1955 with the above symptoms persisting A roentgenogram (Fig 3) revealed a large cystic lesion containing fluid in the right upper lung field He was admitted to Burge Hospital for study The histoplasmin skin test was positive A tuberculin skin test was negative Several sputum examinations were negative for acid fast organisms Bronchoscopy was performed and a stenosis of the right upper lobe bronchus was observed Smears from this area were negative for malignant cells On October 20 1955 right upper lobectomy was performed Sections of the upper lobe showed a



Fig 3

multiloculated cyst the largest measuring approximately 6 cm in diameter The cyst linings were covered with a fibrous exudate Sections of the upper lobe bronchus showed it to be obstructed completely obliterated by fibrous tissue G M S stains revealed numerous organisms identified as Histoplasma capsulatum The patient has returned to his normal occupation as a truck driver

Bronchiectasis associated with pulmonary histoplasmosis and right middle lobe syndromes have been seen on several occasions in the past few years It is our belief that the middle lobe syndromes occur from the adenopathy which destroys this lobe in the majority of cases although in one case very few nodes surrounding the bronchus were found while the bronchi were bronchiectatic and the lobe destroyed by fibrous tissue

Case Report R W a 40 year old white female was admitted to Missouri State Sanatorium on January 6 1956 She stated she had coughed up small amounts of blood in 1945 and 1949 On December 14 1955 she coughed up approximately two cups of bright red blood and was admitted to Burge Hospital in Springfield Missouri After bleeding was controlled bronchoscopy was done on December 20 1955 This revealed extensive bronchiectasis in the left lower lobe and lingula (Fig 4) She was transferred to Missouri State Sanatorium and on January 16 1956 left lower lobectomy and lingulectomy were performed Tissue cultures from the operative specimen were reported positive for Histoplasma capsulatum The organisms were also demonstrated in the tissue utilizing G M S stains She has had no further evidence of hemoptysis and has resumed her activity as a housewife

Two cases have been seen in which the organism Histoplasma capsulatum could be cultured from the empyema fluid thus establishing that this phase of the disease may occur also The empyema in these cases was the result of a large bronchopleural fistula eroding from a large cavitary component and in both of these cases the pathological process took several years to develop Two other cases have been seen in which an empyema was present but in which the organism Histoplasma capsulatum could not be demonstrated In both of these cases however the histoplasmin skin test was strongly positive and the serological titer reached



Fig 1

1 128 In these latter two cases a Schede type of thoracoplasty over the empyema space resulted in apparent arrest of the disease

Case Report H T a 55 year old white male was admitted to Missouri State Sanatorium for the second time on July 13 1956 He had previously been a patient in this institution from January 1950 to May 5 1950 A diagnosis of pulmonary tuberculosis moderately advanced activity undetermined was made at that time although no acid fast organisms were found Over the next few years the patient complained of weakness moderate dyspnea cough and edema of the lower extremities His dyspnea finally became more marked and he was readmitted to Missouri State Sanatorium July 13 1956 X ray at that time showed

marked progression over the previous film of 1950 (Figs 5 and 6) A large air space involving the upper two thirds of the left hemithorax and fluid levels over the diaphragm were present A cavitary lesion was noted in the right apex Repeated sputum examinations were reported negative for acid fast bacilli Cultures from the sputum and the empyema fluid were reported positive for Histoplasma capsulatum Patient was placed on Amebacide and Amphotericin B but did not tolerate this form of therapy On September 19 1956 he was taken to the operating room for exploration and possible thoracoplasty A left pleuropneumonectomy was performed The patient expired on September 29 1956

The above case presents two features of pulmonary histoplasmosis which we feel are of paramount importance The first and probably the most significant is the progressive destructive nature of this disease We do not agree with other authorities who feel that this is entirely a benign disease The second is that the organism Histoplasma capsulatum definitely may be the etiology of an empyema

Histoplasmosis with associated tuberculosis has occurred in

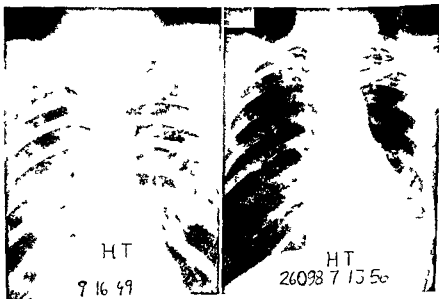


Fig 5 Fig 6

approximately twenty per cent of our cases. This includes the series of coin lesions and also the cavitary phases of this disease. In all cases that had a positive culture for acid fast organisms prior to surgery the sputum became negative for this type of organism when adequately treated with anti-tuberculosis therapy. Even though the sputum becomes negative for acid fast organisms, radiologic improvement has not been manifested in some cases, suggesting a second or underlying disease.

Case Report. I. S., 58 year old white male, was admitted to Missouri State Sanatorium on August 26, 1951. He stated that he had had progressive dyspnea, productive cough, weakness and poor appetite for five months prior to admission. Following his admission to Missouri State Sanatorium repeated sputum examinations were positive for acid fast organisms. The tuberculin skin test and histoplasmin skin test were positive. Serologic studies on September 17, 1951 were reported positive 1:16 in the complement fixation phase. After chemotherapy for his tuberculosis a left pneumonectomy was performed on January 18, 1956 (Fig. 7). Post-resection thoracoplasty was then completed on February 8, 1956. Acid fast organisms were demonstrated in the resected lung tissue and numerous organisms identified as Histoplasma capsulatum were revealed by G. M. S. stains. Patient continues in good health to the present time.

Within the past year we have seen approximately 15 cases which have left us in some doubt as to the viability and virulence of the organisms Histoplasma capsulatum. In each of these 15 cases numerous positive cultures for acid fast organisms have been reported. They all responded to antituberculous drug therapy and became negative prior to having surgical resection. In each of these cases the resected specimen has failed to yield acid fast organisms by stain and culture, yet our pathologists have reported the organism Histoplasma capsulatum when the Gomori methenamine silver stain has been used. Strangely enough, in all of these patients cultures for Histoplasma capsulatum have been negative. The exact role that the organism Histoplasma capsulatum plays in these cases is not fully understood by this author. Further speculation and studies will have to be done before any hypothesis can be given.

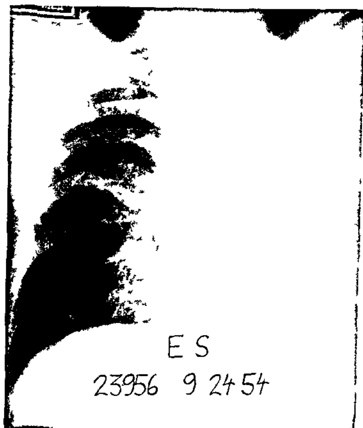


Fig 7

Trends in Treatment

To the present time pulmonary resection has offered the most definitive and successful form of therapy for pulmonary histoplasmosis In spite of this several factors have presented a major deterrent to this form of therapy First a postoperative spread of disease *is feared by surgeons and many will not resect active cavitary* lesions Secondly operating on a disease with an organism resistant to therapy is completely contrary to many sound surgical principals For these reasons as well as control of the more fulminating forms of this disease many medicinals have been tested in the treatment of histoplasmosis

Amphotericin B appears to be the anti fungal agent best suited

in the present treatment of histoplasmosis (16 17) In fact we are in the early stages of surgical therapy with histoplasmosis as we were in the early resection period for tuberculosis when only streptomycin was available

Amphotericin B therapy appears to be promising from a surgical standpoint in several ways First it definitely has been shown that sputum can be converted from positive to negative Most of the patients whom we have followed have reverted to positive status however sometime after the drug is stopped Secondly it appears to us to cause regression in infiltrative lesions although this is hard to evaluate since many infiltrative processes will improve with bed rest alone Thirdly it seems to aid in the control of symptoms particularly cough

Eight patients have had definitive surgical procedures after receiving various amounts of Amphotericin B Six resections and two elective thoracoplasties have been completed Postresection thoracoplasty and closure of a bronchopleural fistula was necessary following a left upper lobectomy and superior segmental resection There was a questionable spread of disease following a second stage thoracoplasty

The major weakness of this form of therapy in our opinion is failure definitely to affect the cavitary and fibrotic disease when it occurs

Case Report H M 51 year old white male was admitted to Missouri State Sanatorium January 5 1958 * In December 1957 he had complained of fever severe cough with marked sputum and exertional dyspnea The patient s symptoms did not improve with penicillin therapy X ray revealed diffuse disease throughout the entire right lung several cavities being present For this reason the patient was admitted to Missouri State Sanatorium (see Fig 8) Following admission to the Sanatorium bronchoscopy was performed January 25 1958 All studies for acid fast organisms were negative Repeated cultures were positive for Histoplasma capsulatum Amphotericin B therapy was started on January 23 and continued until May 5 1958 A dose ranging from 25 mg daily given three times weekly

* This pati nt d [illegible] n an ther hosp tal in Nov mber 19 8 of a [illegible] hr n c pneum [illegible] ia

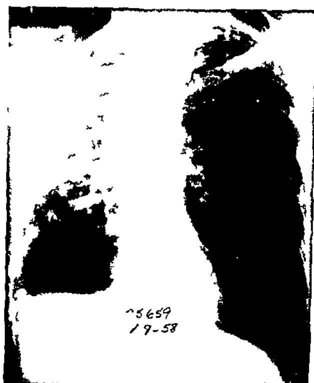


Fig 8

to 70 mg daily three times weekly was used. The patient's sputum converted to negative status around February 4 or three weeks after treatment was instituted. The right lung remained destroyed. On May 6, 1958 a right pleuropneumonectomy was performed. Patient tolerated this surgery well and was discharged May 28 to resume normal activity at home.

As previously stated and shown by this case, we feel that Amphotericin B therapy will convert the sputum in the majority of these cases. At the time of pneumonectomy the right lung was destroyed. On cutting the specimen there were numerous stellate cavities measuring 2 cm in greatest diameter. We do not feel that Amphotericin B would close this type of cavity.

TABULATION OF DATA

Because of the fact that there has been a paucity of resected cases for the various phases of pulmonary histoplasmosis we felt that it would be worthwhile to tabulate our data Furcolow and Brasher (13) in a recent publication stated that only 16 cases of chronic progressive cavitary histoplasmosis had been reported Polk and his group (12) had reported 21 cases of pulmonary histoplasmosis having had definitive surgical therapy

To date we have performed varying surgical procedures on 29 patients presenting various pulmonary pathological processes the etiology of which was Histoplasma capsulatum There have been 4 pleuropneumonectomies in this group The one death which occurred is illustrated in the preceding portion of this text The other 3 patients are living and well

Twenty three patients were treated with pulmonary resection varying from subsegmental resections to lobectomy and segmental resection There has been one death in this series from a pulmonary embolism on the tenth postoperative day This was previously reported by our group Two patients in this series have had relapses or spread of their disease varying from 9 months to 2 years In both of these patients we were able to demonstrate the organism Histoplasma capsulatum in the sputum One has received Amphotericin B therapy the other is being evaluated for this form of therapy

One patient had a left exploratory thoracotomy However because of the extent of the disease which would have required a pneumonectomy with disease in the contralateral lung no definitive surgery was done This patient had received MRD 112 prior to thoracotomy He expired in irreversible shock 60 hours post operatively Autopsy revealed an acute pseudomembranous enteritis with toxic hepatitis and toxic nephrosis Also bilateral pulmonary histoplasmosis was demonstrated

Of the remaining cases having had definitive surgical procedures results have been satisfactory They have returned to normal life

Two patients with positive sputum have had primary thoracoplasties as a definitive form of therapy One patient had received

Amphotericin B until the sputum became negative following which a two stage thoracoplasty was done This patient has subsequently died of a coronary occlusion It is our opinion that this was a bad selection of cases due to extent of the disease and his low pulmonary reserve The other patient done prior to Amphotericin B therapy has remained positive but continues to lead a quiescent life We feel that his symptoms especially cough were reduced by the thoracoplasty Two patients without positive sputum but with serologies varying from 1 64 to 1 128 had also received primary thoracoplasties These two patients have subsequently returned to their homes Both are in the 60 age group and have not returned to work at the present time

It can easily be seen from the above remarks concerning thoracoplasty that few surgeons have had enough experience to evaluate this operation adequately in pulmonary histoplasmosis We have used it as a secondary operation withholding it for the aged debilitated patient who has had bilateral extensive disease Perhaps if thoracoplasty were used in the young group with unilateral localized disease results would be better

In a series of 65 resections for coin lesions over a six year period 27 cases of granulomatous disease have been found proven to be histoplasmosis by demonstrating the organism in the tissue It is interesting to note however in only 6 of these cases has the organism been cultured from the tissue We have found the Periodic Acid Schiff stain and Gomori s methenamine silver stain to be very useful in this phase of the disease There have been no operative deaths in this series and the entire group has returned to normal life

In 4 of these 27 proven cases of histoplasmosis active pulmonary tuberculosis has also been demonstrated in conjunction with the histoplasmosis In 11 of the remaining cases active pulmonary tuberculosis was demonstrated to be responsible for the coin lesion We have therefore treated all patients who are to have surgery for a coin lesion with antituberculous therapy prior to and following resection The interval prior to surgery has varied from 6 weeks to 48 hours Postoperatively the patients demonstrated to have active tuberculosis have been treated with two years of antituberculous

therapy We feel that this has been partially responsible for our having had no bronchopleural fistulae or complications in this series

Twelve cases of pulmonary histoplasmosis in children excluding the epidemic form have been studied in the past 3 years In all of these children there has been a strongly positive skin test and a high titer serological test It has been on this basis that we have made the diagnosis of histoplasmosis since in none of these children have we been able to demonstrate the organism Histoplasma capsulatum in the sputum

There have been 6 right middle lobe syndromes 2 pneumonic processes and 4 infiltrative type lesions In each patient there has been an acute febrile course with marked cough and lassitude In each of these patients the course has been self limited We have used broad spectrum antibiotics in each case but do not feel that this form of therapy aided the course of the disease It is our opinion however that in the exacerbations the antibiotics are helpful in controlling the secondary infection consequently reducing fever and other symptoms

Complete bronchoscopic studies have been performed in 5 of these patients In 4 the middle lobe bronchus was stenotic due to external pressure In none of these 4 cases could active disease be seen in the bronchial lumen Attempts have been made to dilate the bronchus in each patient Two patients followed for over 3 years have improved and the atelectasis in the middle lobe has disappeared In 2 the middle lobe syndrome persists We have elected to follow these children rather than perform middle lobectomy until more definitive knowledge can be gained concerning this type of disease

Within the past year approximately 15 cases have had surgical resection at the Missouri State Sanatorium presenting a somewhat complicated picture In all of these cases the sputum was positive for acid fast bacilli on admission both culture and direct smear After reverting to a negative status on drug therapy resection was done Tissue studies then revealed no evidence of active pulmonary tuberculosis In each of these cases organisms resembling Histoplasma capsulatum were demonstrated with the Gomori methena

mine silver stain In all of these cases cultures were negative for histoplasmosis At this time we are unable to make further comments concerning this since more study and follow up will be needed upon this type of case All are living and well

The remaining cases mentioned in our classification will most probably be discussed in other sections of this text and will not be included in this group since no definitive surgical procedures were done

SUMMARY

A classification based on surgical findings and cases of proven histoplasmosis is presented The indications for surgery in pulmonary histoplasmosis are not definitely established due to the paucity of cases We therefore utilized many of the established indications for resection in pulmonary tuberculosis to guide our selection of cases for surgery with this disease

Surgical resection appears to offer the most definitive form of therapy at the present time Further experiences will be needed before the final evaluation of Amphotericin can be established in the treatment of histoplasmosis

REFERENCES

1 Greer S J Forsee J H and Mahon H W Surgical management of pulmonary coccidioidomycosis in focalized lesions *J Thoracic Surg* *18* 591 1949

2 Cotton B H and Birsner J W Surgical treatment in pulmonary coccidioidomycosis Preliminary report of thirty cases *J Thoracic Surg* *20* 429 1950

3 Melick D W Excisional surgery in pulmonary coccidioidomycosis *J Thoracic Surg* *20* 66 1950

4 Lowry C C Kraeft N H and Hughes F A J Blastomycosis of the lung *Am J Surg* *81* 676 1951

5 Hodgson C H Weed L A and Clagett O T Pulmonary histoplasmosis Summary of data on reported cases and a report on two patients treated by lobectomy *J A M A* *145* 807 1951

6 Forsee J H Puckett T F and Hagman F F Surgical considerations in focalized pulmonary histoplasmosis *J Thoracic Surg* *6* 131 19 8

7 Johnson H E and Batson R Benign pulmonary histoplasmosis A case report with brief review of literature *Dis Chest* *14* 517 1948

8 Bunnell I L and Furcolow M A report on 10 proven cases of histoplasmosis *Pub Health Rep* *63* 299 315 1948

9 Sutliff W D Hughes F A Ulrich E and Burkett L L Active chronic pulmonary histoplasmosis *A M A Arch Int Med* 92 571 1953

10 Polk J W Brasher C A deCastro J and Buckingham W W The surgical treatment of pulmonary histoplasmosis with an evaluation of MRD 112 as a possible adjunct *J Thoracic Surg* 31 118 162 1956

11 Hughes F A Whitaker H W Lowry C C Polk J W Foley F F and Fox J R Jr Resection for mycotic pulmonary disease *Dis Chest* to be published

12 Polk J W Cubiles J A and Buckingham W W The surgical treatment of chronic progressive pulmonary histoplasmosis *J Thoracic Surg* 34 3 3 341 1957

13 Furcolow M L and Brasher C A Chronic progressive (cavitary) histoplasmosis as a problem in tuberculosis sanatoriums *Am Rev Tuberc* 73 609 1956

14 Puckett T F Pulmonary histoplasmosis a study of 22 cases with identification of Histoplasma capsulatum in resected lesions *Am Rev Tuberc* 67 1 3 1953

15 Sweany H C Gorelick D Coller F C and Jones J L Pathologic findings in benign pulmonary histoplasmosis *Dis Chest* 34 119 1958

16 Lehan P H Yates J L Brasher C A Larsh H W and Furcolow M L Experiences with the Therapy of sixty cases of deep mycotic infections *Dis Chest* 3 597-613 195

18 Davis E W Peabody J W Jr and Katz S The solitary pulmonary nodule *J Thoracic Surg* 3 728 771 1956

17 Lehan P H Brasher C A Larsh H W and Furcolow M L Evaluation of clinical aids to the diagnosis of chronic progressive cavitary histoplasmosis *Am Rev Tuberc & Pulm Dis* 75 938 1957

THE PLACE OF HISTOPLASMOSIS IN THE PRACTICE OF MEDICINE

John H Seabury

In 1945 the medical practitioner was little concerned with histoplasmosis considered then to be a rare and fatal disease Within the next ten years the story of coccidioidomycosis was found to be largely true for histoplasmosis In some states it was a more common infection than tuberculosis Most cases were relatively mild or actually asymptomatic Enough of the epidemiology and clinical characteristics had been recorded to permit the alert physician to suspect the disease early in its evolution Skin test antigens and serologic diagnosis became readily available When used intelligently these tools permitted the practitioner to make a reasonably certain diagnosis of active disease

The good general practitioner pediatrician and internist learns about diseases common in or peculiar to the geographic area as soon as he selects his location There are significant differences in geographic distribution of many infectious diseases within the United States Knowledge of these differences is essential to every day practice The ease and frequency of travel has made this part of the patient s history both more important and more rewarding— if the physician is prepared to take advantage of it

Although histoplasmosis is widely distributed its highest incidence in the United States is in those states bordering the Mississippi River basin Physicians in this area should be well acquainted with histoplasmosis

Elsewhere in the United States small focal areas of moderate to high endemicity exist Awareness of the fact that small areas of infected soil may be present in regions supposedly free from the clinical disease may permit the inquisitive practitioner to correctly diagnose an isolated outbreak of virus pneumonia among adjoining farm families

To the physician on the West Coast of the United States histoplasmosis is certainly no problem at present no indigenous cases having been reported It is probably present but still unrecognized in some Western areas More cases will be recognized in the Eastern States as those physicians who have been saying We don t have it here are replaced by those who look for it In middle United States histoplasmosis should be a routine consideration in patients who present with acute or chronic pulmonary disease or granulomas of the skin or mucous membranes

Just how important histoplasmosis is in the practice of medicine depends not only on geography and the natural morbidity and mortality of the disease but also upon the degree to which these latter factors can be modified by treatment and the frequency with which the infection is confused with other diseases of different importance The consideration of some of these factors may help in recognizing the disease and assaying its importance

AREAS OF HIGH ENDEMICITY

In areas of high endemicity such as the Mississippi Valley most cases of histoplasmosis will be primary infections among the young Our present knowledge points to the soil particularly where enriched by chicken droppings as the major source of infection Consequently one would expect the highest incidence among five to fifteen year-old children since this is the age group with greatest exposure to the soil while still non immune Newcomers from areas of low endemicity will also be particularly likely to have primary infections regardless of age

Since primary histoplasmosis is usually mild and self limited is there any reason why the practitioner should make an especial effort to diagnose it? Is it not sufficient to recognize the few in whom the disease progresses chronically or disseminates acutely? There is insufficient knowledge at hand to answer these related questions factually but by combining what is known with conjectures derived from analogy to tuberculosis one can arrive at reasonably probable answers

Primary histoplasmosis in infants seems to be accompanied by serious dissemination more often than in older children or adults

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In 1915 the medical practitioner was little concerned with histoplasmosis, considered then to be a rare and fatal disease. Within the next ten years the story of coccidioidomycosis was found to be largely true for histoplasmosis. In some states it was a more common infection than tuberculosis. Most cases were relatively mild or actually asymptomatic. Enough of the epidemiology and clinical characteristics had been recorded to permit the alert physician to suspect the disease early in its evolution. Skin test antigens and serologic diagnosis became readily available. When used intelligently, these tools permitted the practitioner to make a reasonably certain diagnosis of active disease.

The good general practitioner, pediatrician, and internist learns about diseases common in or peculiar to the geographic area as soon as he selects his location. There are significant differences in geographic distribution of many infectious diseases within the United States. Knowledge of these differences is essential to everyday practice. The ease and frequency of travel has made this part of the patient's history both more important and more rewarding—if the physician is prepared to take advantage of it.

Although histoplasmosis is widely distributed, its highest incidence in the United States is in those states bordering the Mississippi River basin. Physicians in this area should be well acquainted with histoplasmosis.

Elsewhere in the United States small focal areas of moderate to high endemicity exist. Awareness of the fact that small areas of infected soil may be present in regions supposedly free from the clinical disease may permit the inquisitive practitioner to correctly diagnose an isolated outbreak of "virus pneumonia" among adjoining farm families.

AREAS OF MODERATE ENDEMICITY

Relatively more adults will have primary histoplasmosis in areas of moderate incidence than in areas of high endemicity. Except for rare focal outbreaks coming from highly infected caves, old chicken yards and bat roosts, there is not likely to be acute (diffuse epidemic) pulmonary histoplasmosis in the area. The same complications of primary infection can be expected as in areas of high endemicity, but since the disease is less common, misdiagnosis will be more frequent.

Symptomatic primary and chronic reinfection histoplasmosis produce more morbidity in areas of moderate and low endemicity than they should. Failure to suspect the diagnosis and improper treatment is quite common. Even in areas of high endemicity, surveys of tuberculosis sanatoria reveal a significant number of patients who are being treated without bacteriologic confirmation. Many of them have been sent in by practitioners who have initiated antituberculous therapy on the basis of roentgenographic evidence or signs and symptoms without serologic or cutaneous testing for histoplasmosis, blastomycosis or coccidioidomycosis.

Confusion of primary and chronic progressive pulmonary histoplasmosis with tuberculosis may result in needless confinement, concern and prolonged misdirected antimicrobial treatment. Since specific antimicrobial therapy is available for both tuberculosis and the yeast-like fungus infections, accurate diagnosis has become essential to good practice and may actually assume medico-legal importance.

The practitioner in areas of high and moderate endemicity of histoplasmosis must have either positive bacteriologic evidence of tuberculosis or a positive tuberculin skin test and negative cutaneous, serologic and usually cultural studies for histoplasmosis before he should make a diagnosis of tuberculosis. This obviously is not applicable to the initiation of treatment for suspected miliary or meningeal tuberculosis.

These ethical and legal implications are sufficiently important to stimulate practitioners to demand better clinical pathologic mycology from their hospitals (which, by and large, are grossly inadequate in this field) and to become fully cognizant of the indi-

The situation may be likened to primary tuberculosis in infants *It is probable that histoplasmosis in infants should be treated* antimicrobially rather than expectantly

Acute (epidemic diffuse) pulmonary histoplasmosis is associated with massive inhalational infection and is more likely to be *seen in areas of high endemicity where heavily infected soils are* present (caves old chicken coops and yards bat roosts) Even though rarely fatal this form of the disease produces severe illness and often prolonged asthenia It is desirable in my opinion to treat this clinical type of histoplasmosis with specific antimicrobials if the diagnosis can be suspected early Treatment should be instituted before mycologic or serologic diagnosis is possible so that suspicion and careful history taking are essential This form of histoplasmosis needs study to determine whether it can alter significantly the function of the lungs and liver *Extensive fibrotic* lesions may remain in these organs after the infection has subsided Healing by fibrosis may be more detrimental to visceral function than recovery with multiple calcified areas of necrosis

Primary infection in children and adolescents other than the acute pulmonary (epidemic) form is commonly unrecognized because of its mildness Those with moderately severe primary infections are usually correctly diagnosed or thought to have either *tuberculosis or virus pneumonia*

The primary infection in children and adults needs long term statistical study relative to the frequency and severity of complications and the advisability of restricting activity or antimicrobially treating the active phase of the illness It is known that the primary infection may be complicated by obstructive atelectasis which may give rise to bronchiectasis or lung abscess The frequency of these complications is not known nor is the effect of limiting activity in their prevention The mediastinal nodes are sometimes involved and serious obstructive mediastinitis may result *It is probable that* primary infections accompanied by extensive lymphadenopathy in the mediastinum or by compression of a bronchus should be antimicrobially treated It is likewise probable that symptomatic care *alone is sufficient* for most primary infections of children and adults There are sufficient cases in areas of high endemicity to permit statistical study of these problems

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tion which persists during weeks of observation who has negative bacteriologic and mycologic studies of the sputum and fasting gastric contents and definite cutaneous reactions to tuberculin and histoplasmin is a real and not rare challenge. In such a dilemma

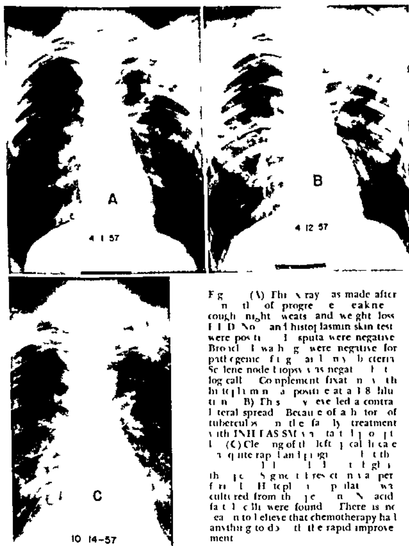


Fig [illegible] (A) This x ray was made after [illegible] of progressive weakness, cough, night sweats and weight loss. [illegible] and histoplasmin skin test were positive. [illegible] sputa were negative. Bronchial washings were negative for pathogenic fungi and [illegible] bacteria. Scalene node biopsy was negative [illegible]. Complement fixation with histoplasmin was positive at a 1:8 dilution. (B) This [illegible] revealed a contralateral spread. Because of a history of tuberculosis in the family, treatment with INH PAS SM was started [illegible]. (C) Clearing of the left apical disease was quite rapid and progressive [illegible]. Segmental resection [illegible]. Histoplasma capsulatum was cultured from the specimen. No acid fast bacilli were found. There is no reason to believe that chemotherapy had anything to do with the rapid improvement.

cations methods and materials needed for the laboratory diagnosis of the mycoses Fig 1 illustrates the consequences of inadequate laboratory diagnosis

Confusion or uncertainty of diagnosis cannot always be prevented The patient who presents with apical pulmonary infiltra

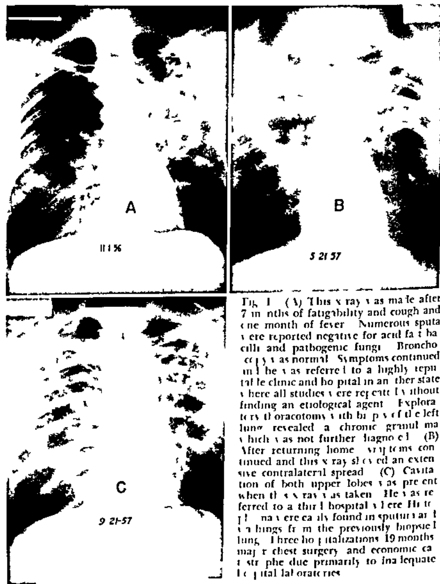


Fig 1 (A) This x ray was made after 7 months of fatigability and cough and one month of fever Numerous sputa were reported negative for acid fast bacilli and pathogenic fungi Bronchoscopy was normal Symptoms continued and he was referred to a highly reputable clinic and hospital in another state where all studies were repeated without finding an etiological agent Exploratory thoracotomy with biopsy of the left lung revealed a chronic granuloma which was not further diagnosed (B) After returning home symptoms continued and this x ray showed an extensive contralateral spread (C) Cavitation of both upper lobes was present when this x ray was taken He was referred to a third hospital where Histoplasma were easily found in sputum and in lungs from the previously biopsied lung Three hospitalizations 19 months major chest surgery and economic catastrophe due primarily to inadequate hospital laboratories

SPECIAL PROBLEMS COMMON TO ALL AREAS OF HISTOPLASMOSIS

Many of the clinical problems associated with histoplasmosis owe their existence to failure of clinicians to suspect the presence of the disease Familiarity with clinical forms of histoplasmosis will do much to increase suspicion and orderly attempts to establish the diagnosis In addition the practitioner needs to refresh his knowledge of microscopic pathology not for the purpose of supplanting the pathologist but rather to properly interpret the pathologist s report and to argue with him when indicated

Whatever his formal knowledge may be the American practitioner seems to associate the histopathologic designation chronic granuloma with tuberculosis foreign bodies and perhaps syphilis Forgotten are the many causes of chronic granulation tissue Instead of representing a diagnostic challenge this simple tissue diagnosis is likely to be accepted as an answer Unfortunately the presence of multinucleate giant cells and especially caseation necrosis is often sufficient to lead the pathologist to unwarranted assumptions The practitioner must remember that he like the surgeon in the operating room is responsible for the diagnosis and treatment of his patient He must know what is required for a diagnosis and he must find out if these requirements have been met In their efforts to be helpful to the clinician both pathologists and radiologists often report that a process seen by x ray or in the microscope is suggestive of tuberculosis sarcoidosis or some other specific process Too many practitioners assume that these suggestions are fairly specific diagnoses and proceed accordingly

No matter what the histologic pattern of a granuloma may be search for etiologic agents should be undertaken It is best when the clinician insists that a generous portion of a biopsy be separated before fixation and sent on sterile gauze or in sterile saline for cultural and animal inoculation Diagnosis can be made sometimes by special staining but the tendency to rely on tissue diagnosis without culture or animal inoculation should be resisted vociferously and firmly

Another American fallacy is the dictum that a single diagnosis should be made to answer all the findings of the history and physi

the practitioner may resort to a therapeutic trial This is sound practice but it is quite fallacious to attach any diagnostic importance to a favorable response *Fig 2 illustrates this problem*

AREAS OF LOW ENDEMICITY

At the present time the extent and position of areas of low endemicity is not well known Until the epidemiology of histoplasmosis is more completely studied one cannot be certain of the importance of animal reservoirs either in the infection of man or in maintenance of and extension of focal areas of soil contamination The apparent similarity between histoplasmosis and coccidioidomycosis would make one believe that wherever animal histoplasmosis is found human cases will be discovered This does not imply any particular correlation between the density of animal and human infection At any rate present knowledge suggests to many students of histoplasmosis that areas of low endemicity will be characterized by few focal points of soil contamination rather than by a generally low soil count or scarcity of animal infection

In areas of low endemicity few physicians will encounter clinical cases of histoplasmosis and a poor diagnostic record is to be expected The cases most likely to be recognized are those chronic progressive adult cases with granulomas of the mucous membranes or other biopsiable sites Infection may occur at any age since immunity is not acquired by childhood primary disease Cases may be multiple within a single rural family if heavy exposure at a focal point occurs By seeking such a history (e g clearing away an abandoned chicken coop) the alert physician may find the disease in supposedly uninfected areas The pathologist who carefully studies all biopsy and necropsy material of a granulomatous nature with special stains is likewise in an excellent position to uncover new areas of histoplasmosis

Fortunately for medicine there are many obsessive and compulsive physicians who always do skin tests with histoplasmin blastomycin and coccidioidin rather than just tuberculin If a positive reaction is investigated new areas of infection may be found and in areas of low endemicity a focal point may be uncovered

Such patients may improve during the first few days of bed rest and antibiotic therapy, the improvement generally being attributed to the antibiotic. The initial improvement with supposedly specific treatment frequently gives the practitioner a feeling of confidence in the diagnosis. Indeed, when the subsequent course of the patient is not as satisfactory, reculturing may result in the judgment that the patient now has superinfection by bacteria insensitive to the antibiotic prescribed for the initial illness. Such errors are not entirely avoidable but may obscure the true nature of the infection. Simple microscopic study of sputum smears stained by the Gram or Wright methods prior to the institution of antimicrobial therapy would prevent some of these mistakes. Pyogenic bacteria are associated with an abundance of polymorphonuclear leucocytes in the exudate when they are producing disease; a definite predominance of the offending organism can be seen in stained smears during the early stage of clinical symptoms. The presence of many macrophages, lymphocytes and alveolar cells with only a small or moderate number of polymorphonuclear leucocytes is unlikely to be associated with acute staphylococcal, pneumococcal or streptococcal disease. Unfortunately modern practice rarely includes microscopic examination of the sputum by the physician himself. The cellular exudate in the sputum of patients with chronic progressive cavitary histoplasmosis frequently contains a fairly equal distribution between polymorphonuclear and so-called round cells.

In recent years there has been a growing interest in the solitary, well marginated, round or oval density in the lung fields. When of appropriate size, these are often referred to as coin lesions. This type of lesion is rarely diagnosed by any type of laboratory study of the sputum or bronchial washings. The etiology of these lesions is quite variable. In some of the reported series, carcinoma has been a very important cause, whereas in others it has been much less prominent. Histoplasmosis and other mycotic infections may produce single or multiple coin lesions. Histoplasmomas rarely contain culturable Histoplasma at the time they are removed and are generally considered benign. One instance of central liquefaction and concomitant spread is known to the author.

Coin lesions that are due to chronic infectious granulomas

cal examination providing the majority of the laboratory studies are consistent Such a statement tacitly assumes that each disease is clinically unlike all other diseases and disregards the fact that disease of one system or organ predisposes to other diseases of the same tissues The practitioner may be content with a single *proven* diagnosis so long as the course and response to treatment is as expected Some errors will be made but these are pardonable Histoplasmosis and other mycoses should be considered when the patient s course or response to treatment is atypical of the diagnosis This applies to patients with infection neoplasia or collagen disease Mycotic infection occurs with increased frequency among diabetics just as does tuberculosis Tuberculosis and a deep mycosis are common companions in my experience

There are two situations which are particularly challenging to the practitioner the appearance of a mycosis during prolonged antibiotic treatment for another disease and confusing an acutely progressing systemic mycosis with bacterial infection

Although Candidiasis appears to be the most frequent fungus infection complicating prolonged antibacterial treatment histoplasmosis should be watched for in its endemic areas This situation is most likely to arise in debilitated patients who have had dormant primary disease The endogenous reinfection is likely to be insidious and mistaken for either an exacerbation of or the development of resistance by the primary disease The physician should be particularly alert to the possibility of this complication during the management of children with chronic pulmonary infections and chronic cystic fibrosis This complication is almost equally dangerous in the antibiotic treatment of the aged

Acute pulmonary histoplasmosis and progressive febrile histoplasmosis may be diagnosed as bacterial infections at least during their early evolution The presence of constitutional manifestations and pulmonary findings suggestive of an infectious process leads to culture of the throat and sputum If a bacterial pathogen is reported such as the Pneumococcus Hemophilus influenzae or staphylococci and streptococci the illness may be attributed to the bacterial pathogen Unfortunately the presence of such bacteria do not necessarily indicate that disease is being produced by them

persistent in seeking the cause of granulomas and chronic pulmonary disease may derive much satisfaction from correctly diagnosing and treating a disease which is rare in his community

In all areas there must be continued education of all physicians in the proper care and disposition of the biopsy Dentists oral surgeons otorhinolaryngologists dermatologists gynecologists and general practitioners must be urged to biopsy persistent lumps bumps and sores and to see that culture or animal inoculation is a part of the study of the biopsy Pathologists and hospital administrators frequently resist animal inoculation as a diagnostic method on the basis of economics and the difficulty of the animal care Nevertheless inoculation of hamsters or young mice is the single laboratory procedure most likely to result in a definite diagnosis of histoplasmosis Clinical pathologists and bacteriologists must improve their own knowledge of medical mycology and greatly improve the training of the Registered Medical Technologist Etiology cannot be established for all granulomatous processes but the up to-date pathologist can inform the practitioner of (1) the facilities available locally and through the various Health Services and (2) the materials which must be submitted Only two things are necessary an enlightened pathologist and a conscientious practitioner willing to ask

usually acquire laminar or granular deposits of calcium with aging Despite the fact that calcium may occur in neoplastic coin lesions this is so rare as to be of little practical importance However calcium deposits in lung anterior or posterior to the coin lesion itself may appear to be within it in a routine chest x ray It is my own practice to consider all coin lesions which show definite calcium depositions by laminography as non neoplastic in origin I believe that this is adequate grounds for observing such lesions rather than removing them surgically

The early development of histoplasmomas may be accompanied by an elevated complement fixation titer and a positive histoplasmin skin test During quiescence the complement fixation reaction probably disappears quite rapidly although there is insufficient statistical study of this process to permit definite statement Again in my own practice a coin lesion in children or young adults with a positive histoplasmin coccidioidin or blastomycin skin test is kept under periodic observation even though it does not contain calcium Fortunately most histoplasmomas in middle aged or elderly adults contain calcium

The place of histoplasmosis in the practice of medicine will depend primarily upon geography and specialty Physicians should be familiar with the disease in areas of high endemicity and correct diagnosis should be made as a rule by general practitioners pediatricians and internists In areas of high endemicity the frequency of coin lesions demands that the urgeon be familiar with this form of the disease

In areas of moderate endemicity the pediatrician and internist should arrive at the diagnosis in most patients with active disease These specialists should be correct in their presumptive diagnosis of most chronic lesions and symptomatic primary infections The general practitioner should know that the disease occurs within his area and how to look for it For him proper diagnosis or suspicion resulting in later diagnosis by others should be both rewarding and stimulating

In areas of low endemicity correct diagnosis is likely to remain principally with the pathologist and bacteriologist who is adequately trained in mycology However the practitioner who is

In the yeast phase there is considerable variation in size but the average is 1½×2 microns × 3×3½ microns. Under different conditions there may be a great variation in size. One pole is usually pointed where buds form or where buds are prone to appear but buds may form at either pole or sometimes apolar around the walls. Small refractile oil droplets are present in the walls with small refractile granules in the cytoplasm undergoing Brownian movement. The nucleus is usually a crescent shaped mass around the bud at the periphery. When it divides part goes into the bud and part remains in the body of the yeast.

The nature of African histoplasmosis is described and has been designated as H. Duboisii. This strain or variety is occasionally found here. Usually the forms are larger than the common type of H. capsulatum.

The author goes into the many physiological reactions of the fungus with regard to sources of the elements in metabolism, production of acid, gas and the H ion concentration under different circumstances.

The chapter by Dr. Emmons begins with the pertinent statement that the sporadic occurrence of histoplasmosis without evidence of contagion suggested that there might be a reservoir in nature. He confirmed this suspicion by culturing H. capsulatum from soil and demonstrating macrocondidia in the first two positive soil specimens. The author describes the technique of isolation from the soil.

In an area of northern Virginia where there was a high incidence of positive skin reactions and calcifications in human beings on the x-ray, 3½% of the rats were found to have histoplasmosis and 14% of healthy dogs and cats were found with H. capsulatum in their lymph nodes but without demonstrable lesions. The first samples of soil found positive were found at the entrance of a rat burrow near a chicken coop which pointed to the association with old chicken droppings rather than to the rats as the source of the fungus. Further studies revealed that fresh chicken manure in well kept chicken houses did not reveal any fungus. This indicates that the time factor, drying factor or something else enters into the process.

SUMMARY

For the convenience of the casual reader as well as for those who wish to select certain chapters for study it was deemed expedient to summarize briefly even if incompletely the chapters which do not have a summary prepared by the author

The first two chapters do not need an extensive summary The introduction by Dr Meleney is a combination of scientific data history and personal experiences in the early development of the knowledge of histoplasmosis An interesting part is a brief history of Dr Darling s life with many personal over tones There is naturally some overlapping with Dr Baum s chapter on history but there are so many interesting personal touches that the slight duplication is an attraction rather than objectionable

The chapter by Dr Baum on the history of histoplasmosis needs no further comment as all is said and well said in the text

Dr Pine s presentation of the morphological and physiological characteristics of H capsulatum is remarkably complete He has described the dual phase of the microorganism and tells how to grow them The mycelial phase grows best at 25 30°C on blood media but tuberculated spores form best on Sabouraud s glucose agar Mycelial growth does not grow well at 37° at which temperature the culture tends to be transformed into the yeast phase

The various forms that appear due to different media a difference in H ion concentration temperature and age are between rather wide limits Some are very large and bizarre others are small and less conspicuous The macroconidia form at room temperature on mycelial projections and average from 5 15 microns in diameter and usually become tuberculated characterized by many short hair like protrusions from the walls of the spore The microconidia are only 2 6 microns in diameter and they may form from large or small hyphae They are not tuberculated The microconidia may develop into the macroconidia The tuberculated spore is accepted as the best means of a certain diagnosis of histoplasmosis

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In an area of northern Virginia where there was a high incidence of positive skin reactions and calcifications in human beings on the x-ray, 31% of the rats were found to have histoplasmosis and 14% of healthy dogs and cats were found with H. capsulatum in their lymph nodes but without demonstrable lesions. The first samples of soil found positive were found at the entrance of a rat burrow near a chicken coop which pointed to the association with old chicken droppings rather than to the rats as the source of the fungus. Further studies revealed that fresh chicken manure in well kept chicken houses did not reveal any fungus. This indicates that the time factor, drying factor, or something else enters into the process.

There were reported many other sources of H capsulatum both in animals and in the soil Pigeon bird and bat manure were productive of *positive results Various types of soil and organic* material as found in old silos river bottoms dirt floor of meat houses and caves were found to contain H capsulatum Other possible factors are prevailing winds river basins and valleys The author points out the biological advantage of a free living resistant saprophytic form that resists heat cold and drying over the delicate and sensitive parasitic form that is extremely vulnerable to these various factors

In the chapter on geographic distribution Ajello establishes the fact that histoplasmosis has been reported from every large region of the world except Russia and China Although the greatest incidence has been found in central United States there is no certainty that other regions of the earth may not also be heavily seeded with the fungus providing suitable search is made for it

The author tabulates the reports of findings of H capsulatum in soil and in wild and domestic animals in widely scattered parts of the world although most of the reports are from the United States

He also cites references to many proven cases outside the United States and Canada

The chapter on laboratory diagnostic procedures by Larsh reveals how involved the finding of H capsulatum has become Even with the most meticulous technic isolation of the fungus is not always achieved

Culture methods for the first attempt at isolation include blood *media several of which are available and Sabouraud s It is most* essential that antibiotics including penicillin streptomycin chlor amphenocol and actidion be used to control the secondary con taminants that infest most specimens

The tissue phase or yeast phase as produced in animals is necessary for some strains where there are few cells or where there is uncontrollable contamination By use of one of several accept able blood media the yeast phase may be recovered and it then may be re inoculated back on other media or in HeLa cells

The appearance of the colonies on the various media are

described as well as the use of various animals for inoculation which are required for best results These methods are well presented

The use of stains the polariscope the fluorescent antibody technic aid in identifying the yeasts when they are found

On the problem of immunity against histoplasmosis Salvin states that a sublethal infection of H capsulatum will induce protection in animals such as mice guinea pigs and dogs although the basis of the protection is not yet known The immunizing infection does not eliminate the pathogen but restricts its growth especially in certain organs

Evidence points to the fact that resistance develops in human beings also Epidemics of the disease have been noted to occur in areas of low skin reactivity in people coming from low sensitivity levels or in people with negative skin reactions as shown by conversion from negative to positive histoplasmin reaction during the epidemic

Mice vaccinated with killed yeast cells withstood infection even cerebral infections better than the controls Living cells protect better than dead cells The protective power seems to lie in the cell wall and not in the protoplasm fraction of the cells Precipitins and complement fixing antibodies do not play any role in resistance

Humoral antibodies could not be shown to play any role in resistance although more extensive work is needed to bear this out There is no evidence that agammaglobulinemia causes any increase in the susceptability to yeast infection

The mechanism of increased resistance may have some relationship to the phagocytosis as shown by use of P32 laden cells There is probably an increased capacity of its macrophages to engulf and thus inhibit the growth of the fungus

There is a definite natural resistance as shown by the great increase in serious infections in infants much less in old age and lowest in the teen age period There is need for research in attempt to develop an effective vaccine against th disease

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Dr Tenenberg s article on serological reactions has a complete summary which may be referred to (p 168)

The histoplasmin reaction as given by Drs Palmer and Edwards reveals what a valuable tool it is for finding infections by H capsulatum They give the methods of preparation standardization stability dosage and means of applying histoplasmin There seems to be a considerable amount of cross reaction with coccidioidin much more so than a cross reaction of coccidiomycosis with histoplasmin Attempts at isolation of the active principal thus far have only been partially successful

The histoplasmin reaction is much like that of tuberculin It makes its appearance in four to six weeks after infection and seems to last for years and decades

For an idea of the location of an endemic area of the world the map in Fig 1 (p 198) should be consulted Histoplasmin sensitivity is highly prevalent in parts of America with scattered low prevalence areas in some regions in Africa and Southeast Asia

A more detailed map of the United States is shown in Fig 2 As others have mentioned the central part of the country especially along the big rivers contains the highest incidence of skin reactivity to histoplasmin

Geographic location is of far more significance than other factors such as race sex and occupation There is a slightly higher incidence in males over 12 years than in females higher in whites than in Negroes and higher in rural than in urban populations

The authors point out the difficulty in separating out infected from non infected on the basis of the size of the particular skin reaction especially in the midzones where the reaction may be either positive or negative The x ray is some help in indicating infectivity by the presence of calcifications In the study of students shown in Fig 1 14% of students having a reaction of 6 mm or more have pulmonary findings while only 0 3 of 1% have pulmonary findings with reactions less than 6 mm They show also how the cross reactions with coccidiomycosis casts a doubt on many of the statistics for histoplasmosis

The methods of staining have been well given by Drs Mowry and Frenkel The authors go into the development chemistry and

tion by the air The resistance to drying heat and physical conditions perpetuates the survival of any particular fungus Some fungi produce antibiotics which help to protect the species Weather changes such as temperature and humidity affect the life and spread of fungi of many types Localized existance of certain fungi are known perhaps the most noted to date is coccidiomycosis

H capsulatum does not produce a great number of large tuberculated spores but the small spores are produced in infinite numbers and are viable for long intervals in the soil The latter are small enough to enter readily into the respiratory tract going down as far as the alveoli

The infection rate along rivers and valleys is an important characteristic of H capsulatum as well as the zonal localization Certain soils notably the red yellow podzolic soils have been proposed by Zeidberg as a contributing factor to an increase in H capsulatum but there are many exceptions to this soil theory Emmons has suggested that certain animals may cause the limit of spread of the disease because of their restricted location Birds have been thought of but so many birds migrate that it is hard to accept this theory Water and rivers have been suggested as a means of spread and the growth on certain plants but nothing has yet been definitely established Bird manure is different since 88% of epidemics have been related to this possible source of the parasite

The common denominator for growths of the fungus has been found to be an optimum temperature and a relatively high humidity The most heavily involved area co incides with the greatest number of days of the year between 60 and 90 temperature in region having relatively high humidity

The relationship of winds especially tornadoes to the spread *of the disease as in coccidiomycosis seems to have some merit* but a more important feature is what the author considers a source point such as chicken coops pigeon lofts bat roosts etc These *source points* correspond with many surveys that have been conducted

Many of the infections of the body tend to begin in the lung but a few *gastrointestinal infections have been reported*

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technology of the various stains for the demonstration of fungi especially H capsulatum in tissues They point out the fact that this fungus is more difficult to demonstrate under most conditions than ordinary fungi

They trace the development of the various methods most of which are based on the old Schiff reaction of long ago They credit Lillie McManus Hotchkiss & McManus Bauer Gridley which is a modified Bauer method Kligman most of whom have had some role in the development of the PAS stain or other stains of a like nature The two most common reducing agents have been Periodic Acid and Chromic acid As with most others who have worked in this field they emphasize the superiority of the methenamine silver method originated by Gomori for mucin and later applied to yeast by Grocott The technical details of the various stains particularly the latter are given with great minuteness They undoubtedly will serve a good purpose for those who are not familiar with the method

The authors describe the differentiation of other common fungi from H capsulatum

The chapter on pathogenesis of histoplasmosis in animals by Procknow reviews the literature on the subject and reports his own studies concerning the changes in the macroconidia that take place on being inoculated into the lungs of mice The author states that most infections are considered to be by the respiratory tract due to an inhalation of spores which are converted to the yeast forms in the lung and become disseminated by the blood stream to organs rich in reticulo endothelial cells Such organs include the liver spleen bone marrow and adrenals

The author reports the first reaction as described by Brandt The first noticeable effect was a polymorphonuclear leukocytic invasion By 48 hours the macrophages had become predominant By the sixth to the tenth day a granulomatous lesion with reticulo endothelial elements predominated Only a few polymorphonu clear leukocytes lymphocytes plasma cells and Langhan giant cells remained Fibrous tissue later surrounded the area Healing had begun by the 12th day with an increase in plasma cells fibrocytes and a diminishing number of reticulo-endothelial cells Yeast cells became absent by the 31st day

Pure mycelial inoculations were converted to the yeast phase within one week. Inoculations of chlamydospores resulted in phagocytosis by multinucleated giant cells during the first few days. Reticulo endothelial hyperplasia was present. Some free spores were surrounded by polymorphs and most of the spores showed degeneration. After a week the chromatin of the spores became re arranged to form yeast cells. The cell wall ruptured and the free yeast cells were engulfed by macrophages.

The experimental work is given by all routes of inoculation in various animals.

The author's own work traced the spores inoculated intranasally into the lungs of mice from the large chlamydospores to the yeast forms. Within four days dissemination had reached the spleen. By two weeks widespread reticulo endothelial dissemination had taken place. The spores were usually small enough to enter the bronchioles and alveoli.

Even after six hours the spores were seen without any cellular reaction but there was a gradual increase of cells, first polymorphs and other cells afterwards. After 15 to 24 hours the inflammation increased but still remained localized. Many spores remained well preserved even after 36 hours. Polymorphs began to degenerate and become replaced by epithelioid cells. By 48 hours there was an internal trabeculation of the spores which became well developed by 72 hours. From three to five days defects appeared in the walls and yeast like material bulged through the defects into the parenchyma. These cells were then phagocytized by macrophages. This process continued until the seventh day when all spores had disappeared.

The pathogenesis of histoplasmosis in man is only partly understood. The beginning of the infection as so well shown by Procknow in animals and the nature of some of the chronic forms are still quite speculative in human infection. The progressive fulminating type originally described by Darling is about the only form that is known from the beginning of the yeast invasion to the end of the process. There is a marked parallelism to the progressive forms of tuberculosis yet there are many points of difference such as a greater tendency to calcification, greater dissemination of calci

fied lesions in the spleen and a general tendency for the lymph nodes to enlarge more than in most tuberculous infections

In the chronic forms of the disease there are several unknown features that have yet to be explained One for example is the presence of typical yeast bodies in encapsulated lesions some of which reveal great age even in bone formation In a few instances of encapsulated and calcified lesions these sheltered yeasts seem to have been responsible for exacerbation of the disease but exacerbation from very old lesions is as yet doubtful

A most enigmatic aspect is the role of small microspores that seem to be residues of a budding in the yeast bodies Many of these small spores range in size from 0 3 to 0 6 microns and sometimes show birefringence as do the larger yeasts They seem to arise from the yeasts usually in the process of disintegration A bud begins to form in the pointed end or some other place around the wall of the yeast and while it is in the process of formation the yeast body is disintegrated and the bud rapidly forms into a microspore which seems to contain elements of the original yeast body The mechanism of this change has been described by Pine in his chapter If these small bodies can be shown to possess viability it may be another means of prolonging the viability of the parasites after the mother cells are destroyed Proof of this theoretical concept is still lacking but it will bear study

The primary lesion as described by Schwarz states that only *recently has knowledge of the primary lesion of histoplasmosis been* described It has been found to conform to Parrot s law of similar adenopathies as does primary tuberculosis The nature of the lesion is also similar to tuberculosis Frequently there are satellites around the main focus It is not known whether the primary lesion *progresses as does primary tuberculosis or not* but it is altogether likely that it does

The author describes in detail the evolutions of the primary lesion of bronchopneumonia phagocytosis of the yeast by histiocytes histiocytic proliferation epithelioid cell tubercles with caseous centers filled with polymorphonuclear cells although the latter are not dominant Many of the minor features are listed as the lesions become older The lesions are seldom small calcium

is usually chalky, stippled, and rock hard, even with yeast present. All of these features differ from those of tuberculosis. There is a systemic dissemination as indicated by calcific lesions in the spleen and to a less degree, the liver. The lobar localization of these primary lesions have been tabulated.

The small focus is otherwise similar to tuberculosis but the large focus differs in that there is complete destruction of all tissues including the elastic fibers in the center with an increase of fibrosis toward the periphery. There is a merging of the center caseous area with a heavily hyalinized capsule around the outside. The large focus may spread by incorporating collateral inflammation and thus tend to explain the concentric rings that are seen in some lesions.

Lymph nodes become more enlarged in histoplasmosis than in tuberculosis. They may cause compression of bronchi resulting in bronchiectasis, atelectasis, or emphysema. They may rupture and produce hemorrhage.

The author is of the opinion that some of the round lesions described by Puckett are primary lesions but probably not all. Multiple primary foci may and do exist as in tuberculosis.

Puckett describes the large round lesion that is frequently found in histoplasmosis. This intrapulmonary nodule is a form that he calls a histoplasmoma in keeping with terms applied to tuberculosis and coccidiomycosis. These lesions are scattered in all lung lobes corresponding to the scattering of primary lesions in tuberculosis. Some, however, may be re-infection lesions.

They vary in size from 0.5 to 3½ cm in diameter. They may be round or ovoid. They are usually subpleural with a heavy round or ovoid, white or yellowish pleural plaque fused to one side of the fibrotic process. There may be satellites in the adjacent pleura. These lesions are sharply demarcated from surrounding lung parenchyma. The author calls attention to a wedge of heavy fibrous tissue that usually extends into the center of the lesion which he terms the "keystone." Concentric formations are common which give the lesion a laminated appearance. Soft centers may be present in the interior of each lesion where the parasites are found. The parasites are brought out by special stains but cannot be grown on the culture methods that are used at present. Artefacts are many

and must be ruled out before any diagnosis is made The incidence of these round lesions vary geographically but in endemic areas they constitute 50% or more of the round lesions that are found on x ray

The general pathology of histoplasmosis is one of the most recent of the many phases of the disease to be described Until the last few years the only lesions known were those resulting from disseminated disease Most of these lesions were in the organs rich in reticulo endothelial cells lymph nodes spleen liver adrenals and so forth At first there was little caseation but later caseation developed In some perifocal areas there were granulomatous lesions with epithelioid cells and Langhan giant cells

In the chronic forms of the disease it is almost exclusively reticulo endothelial and granulomatous type of inflammation with a scarcity of neutrophils plasma cells and lymphocytes Only occasionally are polymorphonuclears found and they possibly are a result of some complication Of the various organs the adrenals are commonly and severely involved in a caseous necrosis The spleen lymph nodes liver bone marrow are also involved with necrosis and granulomatous type inflammation Necrotic lesions may be formed in the lungs kidneys brain and heart valves

The lung lesions are especially important for they are the principal ones in chronic disease much like that in tuberculosis

The origin of the chronic lesion may be from an older focus that has undergone exacerbation as result of a loss of resistance of the host First there is a histiocytic invasion then granulomatous lesions appear with perifocal caseation There may be fibrotic encapsulation in various areas of large infiltrates The central softening is a granular type of caseation with varying amounts of fibrous debris and destroyed phagocytes These cells usually begin to appear in the caseous areas Finally there may be a rupture of the mass into a bronchus with small to large cavities resulting These cavities may first be made up of pockets due to fibrous bands but complete excavation finally occurs to leave a ragged walled cavity which in time changes to a smooth walled or cystic type Most chronic cavitary cases have cysts in the upper part of the lung with a repetition of the process toward the base as time goes on Some cavitary cases may have extremely heavy hyalinized fibrous walls

On the other hand there may develop rapid destruction of the lobe or a lung with cavitary disease usually as a result of some break in the resistance of the host

There may develop a fibroid pneumonitis that is composed largely of granulomatous tissue epithelioid cells with Langhans giant cells The lymph nodes may be especially enlarged to form tumor masses simulating cancer and causing pressure on bronchi which leads to bronchiectasis or other pressure phenomena

Roentgenological findings in histoplasmosis have been so well summarized and illustrated by Dr Silverman that there is no need for any more comment (p 379)

On the clinical types of histoplasmosis Dr Furcolow approaches the subject from a tentative classification of a complete disease spectrum involving both clinical and anatomic features and not just on the basis of clinical illness alone This decision was reached after the author found more than a dozen different clinical classifications no two of which were alike and also because there are estimated to be thirty million infections in the country with only a small percentage of clinical disease The basic difficulty with easy recognition of the disease he says is that the disease spectrum runs all the way from asymptomatic or mild infection through to chronic cavitary disease He also states The disease is not new so it is obvious that the symptoms have mimiced other diseases otherwise it would have been recognized long ago

The classification is into four main groups

(1) Acute pulmonary histoplasmosis divided into asymptomatic and symptomatic The latter may be mild or severe

(2) Chronic pulmonary histoplasmosis

(3) Acute disseminated histoplasmosis which may be benign or progressive

(4) Chronic disseminated histoplasmosis

Other forms recognized are acute cutaneous histoplasmosis bronchiolitis bronchitis (middle lobe syndrome) chronic fibrosis resulting in compression (inflow stasis)

So many different classifications perhaps point up the need for a committee to work on uniform classification as has been done for tuberculosis

Dr Sutliff has given an adequate summary on the diagnosis of histoplasmosis (p 421)

In the differential diagnosis Pinkerton states that the mechanisms of the disease are strikingly similar to those of tuberculosis and coccidiomycosis The most important disease to consider in differential diagnosis is tuberculosis and the finding of the respective agents by culture stain or animal inoculation comes first in the attempts to find the cause of the process Immuniological tests are also valuable The author says It is probable that the great majority of cases even when clinical symptoms are present still escape detection and that in the number of cases diagnosis is proportional to the degree of interest in the disease Again he says The differential diagnosis of histoplasmosis is complex multifaceted somewhat controversial difficult and in some instances impossible

There is an elaborate chart which illustrates the various types of localizations of the disease as well as its progression

The asymptomatic pulmonary calcifications are differentiated by positive histoplasmin and negative tuberculin tests Healed histoplasmosis with calcification must be differentiated also from coccidiomycosis which involve many laboratory clinical and x ray study Clinically active forms have to be differentiated from influenza and other respiratory infections of an acute nature Culture and immunological tests are required to achieve this result More severe types of infiltrates and large lymph nodes patchy pneumonitis and radiating infiltrations that must be differentiated from many different conditions like sarcoidosis pneumonia etc A positive tuberculin and negative histoplasmin and vice versa are extremely important in diagnosing either disease

Finally atypical pneumonia Q fever ornithosis and toxoplasmosis pulmonary fibrosis of many types are diseases from which histoplasmosis must be differentiated Acute diffuse interstitial fibrosis (Hammon Rich disease) berylosis acute alveolar proteinosis alveolar cell carcinoma and other conditions may simulate diffuse histoplasmosis The middle lobe syndrome is frequent in histoplasmosis

Cases with prolonged or intermittent fever or chronic cavitary

disease are confused with tuberculosis It requires all the diagnostic facilities available to find the respective parasites Cystic lung Klebsella infections and other fungus infections bronchiectasis silicotuberculosis or other cavitary disease must be considered and which must be separated from histoplasmosis

The author takes up the coin lesion and differentiates the many diseases in this category then the generalized lesion and the local lesion in the many systemic localizations important of which are the pharyngeal infections

An important problem was that of associated diseases Most common is tuberculosis but also Cryptococcosis and other fungus infections Hodgkin s disease and other lymphomas sarcoidosis allergic diseases and many more are also present

The chapter on medical treatment by Yates Langeluttig and Brasher has an adequate summary in the text to which the reader is referred (p 468)

Likewise a summary of the chapter by Dr Polk on surgical resection will suffice to indicate the place of surgery in the treatment of histoplasmosis and give the reader an indication of its content Although Dr Polk s classification differs from most of the others it is intended purely for surgical use It points up a need for a study of this phase of the problem (p 490)

Seabury states that before 1945 the medical practitioner was little concerned with histoplasmosis considered to be a rare and fatal disease up to that time Now it is different because the disease is more prevalent in some regions than tuberculosis There are enough known methods now of clinical and laboratory procedures to permit a general practitioner to suspect and find the disease early The alert physician is or should be acquainted with the geographic distribution of the disease as well as with the many clinical and laboratory tests that help to identify it The many factors of endemicity are mentioned which will contribute greatly to a better understanding of the disease process for the benefit of the practitioner of medicine

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In regions of moderate endemicity there will be relatively more adults having the primary histoplasmosis than in the areas of high endemicity The diffuse and generalized form is not so common Failures of diagnosis are common Many linger in sanatoriums under the diagnosis of tuberculosis and are treated as such In the regions of high and moderate endemicity the physician should have positive findings of tuberculosis and negative findings for histoplasmosis before making a diagnosis of tuberculosis The areas of low endemicity are poorly outlined at present Resort to skin testing for all granulomas is a safe way to pick up cases in all areas

One of the biggest obstacles to clinicians is the pathologic diagnosis of chronic granuloma Because of the tendency to call it tuberculosis or other chronic process without finding the true etiologic agent Instead of representing a diagnostic challenge this simple tissue diagnosis that is chronic granuloma is likely to be accepted as an answer Cultures stains and animal inoculation should be made without delay and continued if necessary to try to find the real etiologic agent

Seabury states another fallacy of making only a simple diagnosis without looking for other superimposed or the association of other diseases such as histoplasmosis and tuberculosis diabetes and other conditions Prolonged antimicrobial therapy should be suspected of producing not only Candidiosis but other fungus infections such as histoplasmosis especially in children and old people Many other possible errors are cited

He discusses coin lesions their role and differential points to watch He points out that an elevated complement fixation test and

positive histoplasmin skin test are most valuable indicators of histoplasmosis

Finally he says in high and moderate endemic areas practitioners pediatricians and medical internists must make the diagnosis whereas in areas of low endemicity pathologists and bacteriologists trained in mycology must perform that service In dentistry oral surgery gynecology and most other specialties the disease may appear so it behooves everybody to be on the lookout for it

Above all pathologists and bacteriologists trained in mycology should use all culture staining and animal inoculation technics possible when the diagnosis is in doubt They should become mycologically conscious This applies to medical technologists also

Etiology of all granulomas cannot be established but the up to-date pathologist can inform the practitioner of the facilities available and the material which must be submitted for examination Two things are necessary an enlightened pathologist and a conscientious practitioner willing to ask

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